

THE GOLGI APPARATUS

PERSONAL OBSERVATIONS AND A REVIEW OF THE LITERATURE

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TWENTY-TWO FIGURES

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I. INTRODUCTION

It is rather extraordinary that there should be within every cell a structure as conspicuous as the nucleus, and sometimes surpassing it in size, the meaning of which is utterly obscure. One at least of the functions of the nucleus—its rôle in heredity—is known to us. We have fairly definite ideas as to the rôle of the centrosomes, and theories aplenty as to the part played by mitochondrial structures, and other types of granules. But as regards the structure to which Golgi has given the name ‘Apparato reticolare interno,’ we have learned only its appearance, its distribution in different types of cells, and its behavior dur-

ing cell division. One of the most recent papers on the subject—that of Kolster (102)—ends with the statement “These structures undoubtedly have a special significance, but we are ignorant of it.”

The credit for the discovery of this intracellular organelle—if such it be—undoubtedly belongs to Golgi, and a large part of the work, including the working out of a fairly easy and satisfactory technique for its demonstration—has been done by Golgi himself, and by his pupils and co-workers, Veratti, Perroncito, Pensa, Negri, Gemelli, Brugnattelli and others. A number of papers dealing with the same structures have been published by Ramon-y-Cajal, who, indeed claims priority for their discovery over Golgi, and by his pupils, Sanchez, Fananas, Tello and others. Nussbaum, in Prague, has inspired a series of papers by his pupils (Weigl, Polyeszynski, Bialschowska and Kulikowska) dealing chiefly with the appearance of the Golgi apparatus in the ganglion cells of invertebrates. Important papers have been published by v. Bergen, Deineke and by Kopsch, who discovered a new and very simple method for demonstrating the apparatus. The best and most exhaustive general review on the subject is that of Duesberg (48), before the XXVIII Meeting of the Anatomische Gesellschaft at Innsbruck in 1914, and Cajal (31) in his most recent publication ('15) which was not available at the time this study was begun, has contributed a most interesting critical survey of the entire field, and added many new observations.

A whole chapter—largely controversial—is that contributed by Holmgren, whose views and their bearing I shall take up later. In this country only Bensley and Cowdry have made contributions to the subject.

In reviewing the literature of the subject I found that but few workers, with the exception of Cajal and his pupils, had attempted to study the behavior of the Golgi apparatus under experimental conditions. I planned, therefore, to follow the modifications of the structure in the epithelial cells of the rat kidney, which might be produced by autolytic changes, secretory phases and toxic agents. The choice of material was unfortunate. The Golgi apparatus of the cells of the renal tubules

proved to be so atypical and variable in form that it was difficult to draw inferences from variations seen under experimental conditions. One was further handicapped by the difficulties and capriciousness of the impregnation method, as applied to this organ.

In attempting to control the technique many other tissues were studied, and insofar as the observations made differ from those of previous workers, they are given below.

Although I was unsuccessful in the main purpose of my study, it seemed that it might be useful at this time to collate the widely scattered and rather inaccessible literature, and to record my personal observations, insofar as they supplement or are at variance with those of other workers in this field.

II. NOMENCLATURE

Golgi (61), in his original communication before the Med. Chir. Society of Pavia in 1898, suggested the term 'Apparato reticolare interno,' and this term has naturally been adopted by all the Italian workers. Kopsch (103) proposed the term 'Binnennetz' as the German equivalent, but both of these designations are open to the objection that the structures do not appear in all types of cells, nor under all conditions, as a closed net. Ballowitz (4) in 1899 described a basket-like structure about the centrosomes of the cells of Descemet's membrane, and suggested for it the name 'Centrophormia.' Later he recognized the homology of the 'Centrophormia' with the Golgi apparatus, and the term has not come into common use. The 'Nebenkern' of Platner (145) and la Valette St. George (106), and the 'Zentralkapsel' of Heidenhain (68) in the sperm cells have been considered by some as related to or identical with the structures demonstrated by the Golgi technique. The terms, however, are not sufficiently inclusive to apply to the structures described by Golgi. Ramon-y-Cajal and his school who agreed with Holmgren in regarding the apparatus as canalicular in nature—referred to it in their earlier publications as the Holmgren-Golgi apparatus. In his latest review, however, Cajal (31) recognizes the very doubtful identity of many of the structures described by Holm-

gren with those brought out by the Silver methods, and therefore refers to them more justly as the Golgi apparatus. Many of the German workers speak of the Golgi-Kopsch net or apparatus.

Holmgren (90), who believes in the identity of the canaliculi described by him, with the structures put in evidence by the silver impregnation methods, uses the term 'Trophospongium.' Cowdry (42) and Bensley (14) speak of 'canalicular apparatus.'

None of these terms appear to be entirely satisfactory. I shall, therefore, refer to the structures simply as the Golgi apparatus.

III. TECHNIQUE

The earliest studies of Golgi were made with a modification of his well-known silver chromate method. This gave capricious and inconstant results. Veratti introduced a modification, the essential feature of which was a fixation in osmium platonic chlorid mixture. Kopsch (103) in 1902 showed that prolonged immersion in 2 per cent osmic acid would demonstrate structures identical with those described by Golgi.

The two methods now most commonly used are those of Golgi (66) and of Cajal (30), and, for the convenience of those to whom the original articles are not accessible, they are given here:

The Golgi method is as follows:

- I. *Fixation:* Formalin (20%)..... 30 cc.
Saturated solution arsenious acid (1%)..... 30 cc.
Alcohol (97%)..... 30 cc.
6 to 24 hours.
- II. *Silver nitrate* 1%..... 1 hour to several days
- III. *Development:* Hydroquinone..... 20 gm. }
Sodium Sulphite..... 1 gm. } 2 to 3 hours
Formalin..... 20 cc. }
Distilled water ad 1000 cc.
Wash in distilled water, dehydrate rapidly and embed in paraffin or celloidin.
- IV. *Toning:*
Solution 'A' Sodium hyposulphite..... 30 gm.
Ammonium Sulphocyanate..... 30 gm.
Distilled water..... 1000 cc.
Solution 'B' Gold chloride..... 1%
Use equal parts of 'A' and 'B'. Tone to grey tone.

Veratti has devised the following procedure for ridding the preparation of silver precipitate after toning:

'A'—Repeated washing in distilled water.

'B'—Rapid passage through following solutions:

(1) Potassium permanganate—0.5 gm.

Sulphuric acid—1.0 cc.

Distilled water—1000 cc.

(2) Oxalic acid—1%

Wash in distilled water. Counterstain with alum carmine.

The latest Cajal method differs from the Golgi chiefly in the use of uranium nitrate in place of the arsenious acid in fixation. It is given as follows:

I. Fixation: Uranium nitrate—1 gm.	} 9 to 11 hours
Formol—15 cc.	
Distilled water—100 cc.	

II. Wash quickly

III. Silver nitrate—1.5%—30 to 40 hours

IV. Wash quickly

V. Reduce in

Hydroquinone—2 gm.

Formol—6 gm.

Distilled water—100 cc.

Add anhydrous sodium sulphite—0.15–0.25 gm. so that solution has a yellow color.

VI. Dehydrate and embed in paraffin.

VII. Toning:

'A' Sodium hyposulphite—3 gm.

Ammonium sulphocyanate—3 gm.

Distilled water—100 cc.

'B' Gold chloride—1%

Use equal parts.

The addition of 30 cc. of ethyl or methyl alcohol to the fixative is recommended by Cajal ('15), as advantageous in the case of nervous tissue.

As counterstain I have found a dilute Giemsa solution to give the clearest pictures. A 1 per cent methyl-green solution may also be used, and it has been found possible to combine also the Altmann mitochondrial stain, as modified by Bensley.¹⁵

The removal of the silver precipitate with permanganate and oxalic acid must be very carefully controlled, as it is easy to bring about a complete decolorization of the Golgi apparatus as well.

Cajal and others have obtained the most constant results in the tissues of young animals. Because of the rapid occurrence of autolytic changes little confidence can be placed in the results obtained with tissues from human autopsies.

Both the Golgi and Cajal methods are exceedingly capricious; the impregnation is rarely uniform throughout the entire block.

The most delicate and important step in these photographic processes, according to several workers, is the initial time of fixation. Each type of cell has its optimum time of fixation, which must be determined experimentally. In many cells, however, as in the lymphocytes, spermatic cells, glomeruli of the kidney, this appears to vary within wide limits. That, at least, has been my experience, and I have obtained identical pictures with fixation varying from 2 to 12 hours.

The technique most recently advocated by Holmgren for demonstrating his Trophospongium is a fixation in trichlorolactic acid (6 per cent) and staining in a freshly prepared resorcin-fuchsin solution. The 'canals' take a purplish black color. Holmgren also gives methods which show the canals as colorless structures upon a stained background.

IV. THE OCCURRENCE OF THE GOLGI APPARATUS IN VARIOUS TYPES OF TISSUE CELLS

1. *Nervous tissue*

The first clear description of the structures is that of Golgi in 1898 (61, 62), in the spinal ganglion cells of *Strix flammea* (Barn-owl); in the same year, he made similar observations upon the spinal ganglion cells of Mammalia; Veratti in 1898 found the same sort of structure in sympathetic ganglion cells. Since these early papers, the Golgi apparatus has been found to be present in many other types of nerve cells—the anterior horn cells (Golgi (66), Cajal (31)), the pyramidal cells of the cortex (Golgi (65), Legendre (107), Collin and Lucien (37), Soukhanoff (167), Cajal (31)), the Purkinje cells and other nerve cells of the cerebellar cortex (Golgi (66), Cajal (31)), of the olfactory lobe (Cajal (28)), the ganglion cells of insects

(Bialkowska and Kulikowska (19)), of the leech and earthworm (Bialkowska and Kulikowska), Crustacea (Jawarowsky (98), Monti (127), Polenzynsky (146), of cephalopods (Weigl (180)).

The apparatus reaches its greatest complexity and size in the spinal ganglion cells of vertebrates, and these have, therefore, been a favorite object of study. In adult vertebrates there is shown by the silver or osmic methods, a definite network of solid, tortuous varicose fibrils, which vary in thickness with different species. This network may completely or partially surround the nucleus and may be in contact with it in places. Where the threads cross or interlace, there are often nodular varicosities. In some species there is a sort of lobulation, into three or four partially separated skeins, and individual filaments may be given off from the main mass, and apparently end freely in the cytoplasm. In all cases the peripheral zone of cytoplasm is left free; at no point does the network, or any of its branches reach the surface.

Monti (127), in the ganglion cells of invertebrates (crustacea, arthropods and cephalopods) found a simple apparatus in the form of curved filaments, often bifurcating or anastomosing, but not forming a closed reticulum. V. Bergen (10), working with the Kopsch osmium method, upon the spinal ganglion cells of the hedgehog, cat, rabbit, rat, mouse, and hen, found that not all the cells showed a complete reticulum as described by Golgi, some containing only short filaments, rows of granules or ring forms. Some of the filaments contained a central clear space, and these he interpreted as degeneration forms. This variation in the appearance of the apparatus in different cells in the same preparation v. Bergen interprets as indicating the transitory nature of these structures. He suggests that they are developed from granules, which range themselves into filaments, form more complex networks, and finally undergo central liquefaction with the formation of canaliculi. Other recent workers, however, using the newer methods of Golgi and Cajal have not confirmed v. Bergen's theory, and ascribe the variations to defective impregnation.

Cajal (31), like v. Bergen, notes variation (or 'modalities') in the type of net occurring in ganglion cells of the same order and size. He strongly rejects the idea that these variations are due to irregularities in impregnation, since they may be found in adjoining cells at similar depths from the surface.

The question has arisen as to whether the Golgi apparatus is identical with any of the other known cytoplasmic constituents of the nerve cell—namely, the neurofibrillae, the Nissl substance or the mitochondria. It seems quite certain, in spite of occasional statements to the contrary, that the Golgi net is unrelated to any of these structures. The net is not continued into the cell processes, as are the neurofibrillae, and the fibers of the net are much thicker and more varicose. By combining Kopsch's method with Bensley's aniline-fuchsin toluidin-blue stain, as Cowdry (42) has done, the Golgi net, Nissl bodies and mitochondria may all be stained in the same cells, and their independence of one another made obvious. It seems hardly worth while to go further into this discussion.

2. *Tissue cells other than nerve cells*

a. Epithelial cells. The presence of a Golgi apparatus was first demonstrated in the squamous epithelial cells of *Ammocoetes* (Lamprey eel) by Marengi (120) in 1903, and in *Lumbricus* by Ramon-y-Cajal in the same year. Since then it has been found in the corneal epithelium by Barinetti (6) and by Deinecke (47). The net is present in all layers. In the superficial cells the net becomes looser, and often almost entirely surrounds the nucleus, whereas in the rete mucosum, it lies at the superficial pole of the nucleus, and in the cells near the surface, only granular bodies are found. This change, therefore, accompanies the aging of the cells, and is characteristic not only of corneal epithelium, but also of skin, oesophageal mucosa, the epidermis of the ducks bill (Deinecke, Kolmer (100)). Some of our preparations of the mucosa of the renal pelvis show a similar differentiation.

A Golgi net has been found also in cells of the epidermal appendages and glands—in the lachrymal gland by Ancona (3)

and in the sweat and sebaceous glands by v. Bergen (10), by Bizzozero and Bottisella (21) (who picture a net surrounding the nucleus, and also, incidentally, could not demonstrate it in the epidermal cells), and by Tello (172).

Since the first paper of Ballowitz (4) in 1898, a net has been found in the single layered epithelial cells of Descemet's membrane by Totsuka (174), Zawarzin (184), and by Deinecke (47). The net in these cells is of special interest because it very clearly lies in relation to the centrosomes, and because it was discovered independently of Golgi's work by Ballowitz. Deinecke in these cells, also, made a careful study of the behaviour of the net during mitosis.

Numerous observations confirm the presence of a Golgi apparatus in the glandular cells of the gastro-intestinal tract. Thus Ramon-y-Cajal ('03) found it in the intestinal epithelium of lumbricus, and of the guinea pig (27); v. Bergen (10) in the chief cells of the fungus region ('04), Golgi (67) in the gastric and intestinal mucosa of frogs, birds and mammals, in the glands of Brunner and of Lieberkuhn ('09); d'Agata (43) in the gastric epithelium of triton ('10) and in the gall-bladder epithelium of the guinea pig (44); Weigl (180) and Kolmer (100) in the gastric and intestinal mucosa of various vertebrates; Kolster (102) in the chief and parietal cells of the fundus, in the pylorus and in the cells of Brunner glands ('13).

Kolster has made several interesting observations on the behaviour of the Golgi apparatus in the gastric cells. He found that when the chief cells were successfully impregnated the parietal cells were not. He also showed that, by using the original Golgi silver chromate method, it was possible to impregnate a system of endocellular excretory canals in the chief cells of the fundus, and that these differed, both in their topography and in their form from the true Golgi apparatus. He noticed also in the pyloric gland cells that the appearance of the net varied with different phases of secretion. In the resting cells the net was quite dense, the meshes small, the form of the whole mass spherical; while in secreting cells, the net was rare-

fied (aufgelockert), elongated and extended to the basal portion of the cell, in close contact with the flattened nucleus.

Cajal (31) also describes in great detail a cycle of changes corresponding to different secretory phases in the goblet cells of the alimentary tract. The Golgi apparatus during the earlier phases undergoes an increase in size, later the argentophile substance becomes dispersed amongst the globules of secretion, and completely disintegrates—not as Kolster (102) believes, becoming merely compressed against the nucleus at the base of the cell.

The inferences which Cajal draws as to the functional significance of these cyclical changes, will be discussed later.

Before having access to Cajal's paper, I had independently observed similar alterations in the mucous glands of the larynx (figs. 1, 2, 3). It seems to be quite clear that the net, which is more distinct and well-formed in cells during the inactive stage, becomes broken up and distributed amongst the globules of mucus in those cells which are actively secreting. In the course of this process, there appears to occur a real quantitative decrease in the amount of the argentophile substance not to be explained merely by its mechanical disruption, and implying some sort of regeneration of the apparatus, after the cell has discharged its secretion and returned to rest.

A Golgi net has been demonstrated by numerous observers in the epithelial cells of various glandular organs, and I shall limit myself merely to giving a list of these. The Golgi net was described in the thyroid by Negri (130) and by Kolster (102); in the adrenal medulla by Pensa (135) and Kolmer (100), and in the cortex by Pilat (144), by Mulon (128) and by Kolmer (100); in the anterior lobe of the hypophysis by Gemelli (60), and in both glandular and nervous portions by Tello (172); in the pancreas by Negri (130), by v. Bergen (10) and by Kolster (102), Kolmer (100) and Cajal (31); in the dog's prostate by v. Bergen (10) and in the hypertrophied human prostate by Verson (179) and by Taddei (171). Von Bergen claims to have been able to recognize the net in unstained scrapings of prostatic epithelium kept alive for a time in the prostatic secretion.

This appears to be the only recorded attempt to observe the Golgi apparatus in the living cell.¹

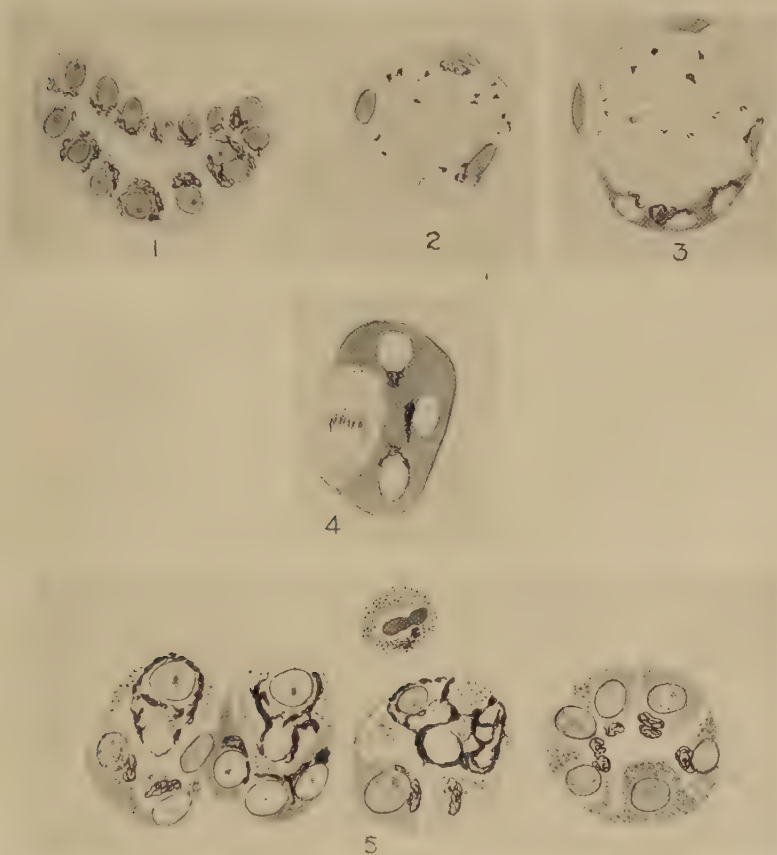


Fig. 1 Tracheal gland, non-secreting. Cajal.

Figs. 2 and 3 Tracheal glands, showing fragmentation of Golgi apparatus during secretion of mucus.

Fig. 4 Small group of thyroid epithelial cells, one in mitosis. Dittokinesis. Cajal.

Fig. 5 Salivary gland of rat. Cajal, anilin-fuchsin-methyl-green. Note relation of Golgi net to Altmann granules.

¹ We also have tried to observe it in growing chick embryo cells in vitro, both by direct light and using dark-field illumination, but without success. Lewis and Lewis (109) likewise report their inability to see structures corresponding to the Binnennetz in living chick embryo cells, nor could they be brought out by prolonged osmic acid fixation.

The net has been found further in the epithelial cells of the epididymis by Negri (130), by Fusari ('08), by Kolster (102) and by Kolmer (100); in the ciliated epithelium of the trachea by Kolster (102); in the choroid plexus by Biondi (20) and in the uterine mucosa and chorionic epithelium by Decio (46) and by Acconti (1).

Negri (130), Kopsch (103), v. Bergen (10), Kolster (102) and Kolmer (100) and Cajal (31), record the presence of a net in the salivary gland epithelium. My preparations show one or two points which I do not find mentioned in their descriptions.

I find in some acini remarkably large, coarse-meshed nets, enveloping the nucleus, joining by stout, varicose filaments with nets in adjacent cells, and not infrequently giving origin to trabeculae which loop about the lumen of the gland. They are thus not confined to a single cell, and anastomose freely one with another.

By counterstaining the Golgi preparations by the anilin-fuchsin-methyl green modification of Altmann's method, one can clearly recognize the independence of the net from the Altmann granules, which are evenly distributed through the entire cell. Indeed, it seems as if these were an inverse relation—that is, those cells in which the granules are poorly marked and absent, show the most conspicuous and clearly defined net, whereas the large cells which are replete with granules, may contain no net at all. Whether this is a constant relation or not, remains to be seen (fig. 5).

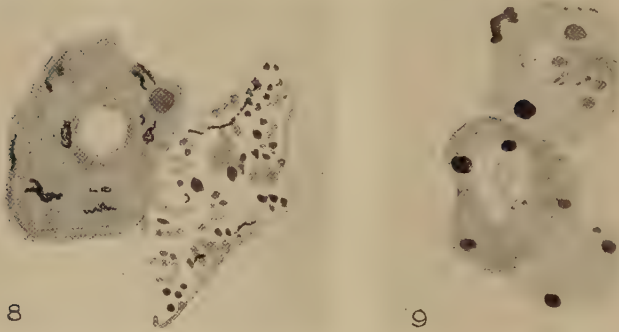
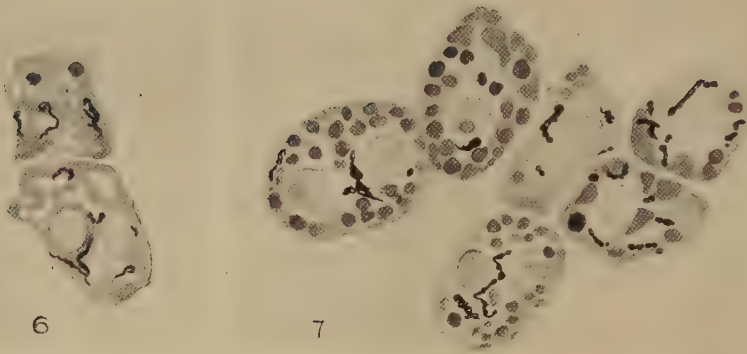
It is surprising that the literature should contain but two references to the presence of a Golgi apparatus in the liver cells. Stropeni (169) is the only one who has succeeded, and he stated that in mammalian livers he obtained only a partial impregnation. With the livers of lower vertebrates, namely frogs and amphibians (Axolotl) he was more fortunate. He found the net to be definitely localized to the portion of cytoplasm between the nucleus and the bile-canaliculi, occasionally sending prolongations into the rest of the cytoplasm; no continuity with the bile canaliculi could ever be observed, nor was there any striking difference in the appearance of the net in fasting or well-fed animals.

Kolmer (100) says that he succeeded but rarely in demonstrating a net in the liver cells. In a new-born cat, the liver cells contained a simple juxta-nuclear net consisting only of several meshes or sometimes of single polygonal nets with one or two long processes. They had no constant relation to the nucleus.

We also have tried repeatedly to find a Golgi apparatus in the liver cells of rats, and have been almost uniformly unsuccessful with the Golgi or Cajal technique. In only one preparation were there found discontinuous curved filaments distributed through the cytoplasm, and bearing little resemblance to the complex reticulum found in other epithelial cells. By prolonged fixation in 2 per cent osmic acid, one may, however, demonstrate very clear-cut intracellular filaments and rows of granules, often curved and occasionally branching, but never uniting to form a definite network (figs. 6, 7, 8, 9, 10, 11). These filaments may lie against the nuclear membrane; in a few instances they appear to join filaments in neighboring cells; in no case do they connect with the bile canaliculi, nor do they appear to reach the surface of the cell. Whether these structures are the homologues of the Golgi apparatus in other cells, I am unable to say with certainty. Their resistance to impregnation by the usual methods implies some chemical variation. They disappear rapidly during autolysis and are absent in cells injured by chloroform poisoning.

Surprisingly few workers also, have concerned themselves with the Golgi apparatus as it appears in the kidney.

Brugnatelli (25), using the Golgi arsenic method has described a net in the cells of the tubuli contorti and of the tubuli recti of the guinea pig, which coincides perfectly with that of other epithelial cells, especially as regards its localization between the nucleus and the lumen of the tubule. In the collecting tubules the apparatus was much more complicated and definite than in the cells of the convoluted tubules, in which it invariably presented itself as small, very simple, almost simulated (i.e., 'accenata'). It seemed, he says, as if the more complex structures here (basal-rods, granules) were harmful to a clear-cut demonstration of the reticular apparatus.



Figs. 6, 7 and 8 Liver cells of rat. Kopsch, 2 per cent osmic acid, 12 days.
Fig. 9 Liver cells of rat, autolysed 3 hours at 37°. Kopsch.
Fig. 10 Liver cells, rat. Cajal.
Fig. 11 Liver cell, rat, vacuolated with localized juxta-nuclear Golgi apparatus. Cajal.

In the glomeruli, the apparatus is reduced to its lowest terms—sometimes appearing as a simple nodule, or as a small figure 8, and always lying adjacent to the nucleus. Brugnattelli gained the impression that the net was restricted to the cells of epithelial origin forming the visceral layer of Bowman's capsule. He is quite wrong in this, as the endothelial cells and the parietal cells of the capsular space also contain an easily demonstrable net (fig. 12).

Barinetti⁶ ('12) describes and pictures a rather complex net in the renal epithelium, and shows its relation to the centrosomes by comparing it with impregnations in which the centrosome is stained by Benda's method. He omits, however, to mention the portion of the renal tubule to which he refers.

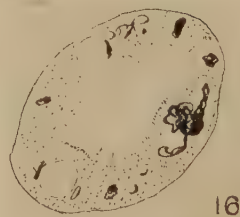
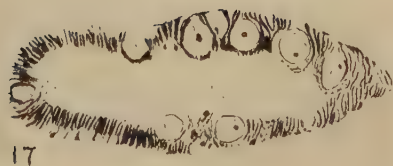
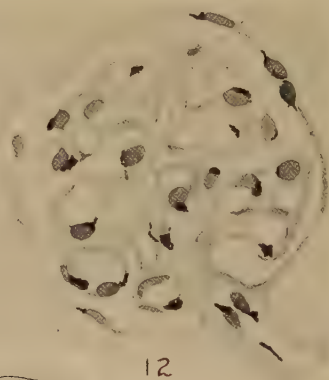
San Giorgi (156) studied the alterations of the Golgi apparatus during experimentally produced nephritis in guinea pigs. He used as toxic agents, uranium nitrate, cantharidin, ricin and diphtheria toxin.

The modifications observed were a splitting up of the filaments or a granular fragmentation without complete loss of the reticular character. Such modifications were most clearly observed in the tubuli recti of the medulla, in which the net, as Brugnattelli showed, is normally more voluminous and complete than in the cells of the convoluted tubules. Close examination showed relation between alterations of the cells as a whole and of the Golgi apparatus. The fragmentation of the net may be marked in some cells of the tubules, whereas others may contain a normal net.

One may criticize San Giorgi's work because of the fact that none of the poisons used produce obvious changes in the cells of the tubuli recti.

Kolmer (100) briefly records the presence of a net in various elements of the kidney, but gives no detailed description.

We have made numerous preparations of rats' kidneys, both of normal animals and of animals in which a uranium nitrate nephritis had been produced. We have varied the time of fixation from two hours (as recommended by Brugnattelli) to twelve hours, without obtaining striking differences. We have also



studied, though incompletely, the effect of autolysis, and found that changes were discernable only after an hour at 37° when the kidney was removed from the body immediately after death. A kidney removed from an animal one hour after death, showed about the same type of structure as the freshly fixed tissue. The apparatus, therefore seems to be somewhat more resistant to autolytic change than the mitochondria, which after half an hour at 37° were broken up into coarse droplets.

As regards the appearance of the Golgi apparatus in the cells of the convoluted tubules, my preparations do not coincide at all with those of Brugnattelli (25) or San Giorgi (156). The argentophile structures in these cells take on the most bizarre and varying forms. One finds smaller and larger droplets or granules, rings or signet forms often ending in a delicate filament, curved threads, uniform in calibre, or with nodular thickenings and varicosities; and larger, more complicated skeins approaching the reticulum described in other types of epithelial cells (figs. 13, 14, 15, 16). The location of these structures with respect to the nucleus is as variable as their form. Their most frequent site is perhaps at either side of the nucleus, sometimes in contact with it; the filaments in general tend to run at right angles to the basement membrane. Sometimes one finds a cluster of granules and short filaments in the supra-nuclear zone, very rarely between the nucleus and the basement membrane. The appearance varies also from one tubule to another in the same preparation; some tubules may show predominantly irregular twisted threads and skeins, others only granulae of uniform or varying size. The appearances are so bewildering in their variety that it is difficult to draw any conclusions, and we are still experimenting with the technique in the hope of getting more constant pictures. We have about decided, however, that the Golgi apparatus in the convoluted tubules is not the

Fig. 12 Rat kidney. Glomerulus. Cajal.

Fig. 13 Rat kidney. Proximal convoluted tubule. Cajal.

Figs. 14, 15 and 16 Rat kidney removed one hour after death. Convoluted tubules. Golgi.

Fig. 17 Rat kidney. Large Henle tubule. Impregnation of basal filaments. Golgi.

clear-cut definite structure which Brugnatelli depicts, but is normally fragmentary and dispersed. We have not been able to prove that these variations in form are correlated with different phases of secretion.

In the Henle tubules, the net is obscured by a very constant impregnation of the Stäbchen. Sometimes, however, we can distinguish a small, very dense, supra-nuclear network, and occasionally filaments are continued along the lateral aspects of the nuclear membrane (fig. 17).

In the cells of the collecting tubules also, a net has been frequently seen, although in the rat's kidney it is a looser and less complex structure than that described by the Italian workers.

In the glomerular cells, both epithelial and endothelial, the small juxta-nuclear net is constantly found, and very sharply defined and striking (fig. 12).

In the uranium nitrate kidney I have made only a few observations which seem worth mentioning at this time.

In the first place, the more complicated filaments and skeins, usually evident in the normal tubules, tend to disappear entirely in the injured kidney. Even those cells, which in sections counterstained with Giemsa, show little or no obvious damage, rarely show structures other than granules or irregular black or grey-staining clumps. In the totally necrotic and desquamated cells, one often finds a single coarse black clump possibly representing the remains of the argentophile structure.

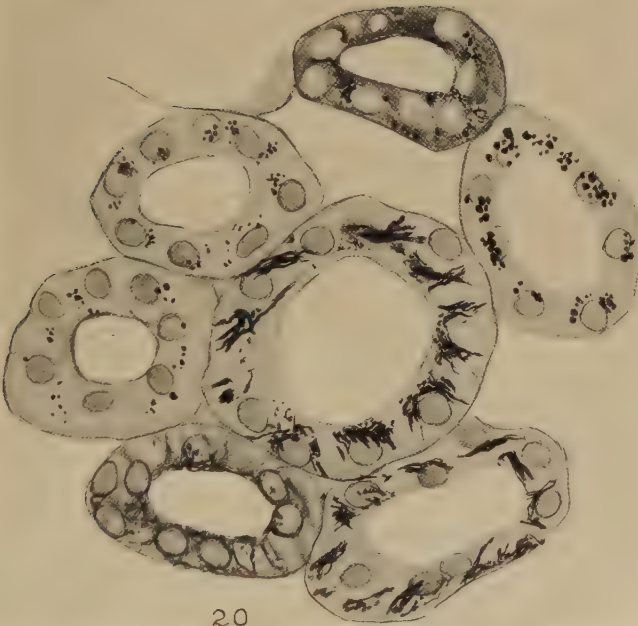
In many of the injured cells I find oval or circular greyish bodies of varying size, many of which contain one or two eccentrically placed black granules (fig. 19). Now starting with these, one can trace transitions both to small solid black granules and to large droplets which have entirely lost their affinity for the silver-stain, taking the eosin of the Giemsa intensely, and resembling in every way the familiar hyaline droplets of degenerating renal cells. These largest droplets accept the acid fuchsin in the Altmann-Bensley stain, but I have gained the impression that they arise from the argentophile droplets rather than from a breaking-up and fusion of the mitochondrial structures.



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19



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Fig. 18 Rat kidney. Two small collecting tubules with delicate supranuclear filaments. Cajal.

Fig. 19 Rat kidney. Uranium nitrate, Nephritis. Proximal convoluted tubule containing hyalin droplets with argentophile granule. Cajal.

Fig. 20 Frog's kidney. Tubules showing various types of argentophile structures. Cajal.

Fahr (52) has also recently made the observation that this type of 'gross-tropfige' degeneration may be produced experimentally in the rabbit's kidney with uranium nitrate, and is disinclined to derive the droplets from the mitochondria of the cell.

The appearances observed in the epithelial cells of the frog's kidney are also very puzzling and difficult to interpret. In the glomeruli, a concentrated juxta-nuclear mass is present in all the cells, identical with that described in the rat's kidney.

In the proximal portion of the convoluted tubule there is found to either side of the nucleus, but rather nearer the distal pole, a small irregular, granular filamentous or ring-shaped mass, which is quite definitely the homologue of the Golgi apparatus of other cells. Such an epithelial cell, cut in a plane parallel to the basement membrane, shows the nucleus surrounded by a ring of discrete masses, which do not form a continuous skein, but are interrupted (fig. 22A).

In frogs injected with trypan-blue this type of apparatus is present in the cells containing the granules of dye, which in general occupy the supra-nuclear zone of the cytoplasm. The dye granules and the argentophile bodies are quite unrelated.

In some of my preparations, the cell boundaries are sharply impregnated by the silver, appearing as delicate black lines. The striated border is also sharply brought out (fig. 22).

In another part of the tubule, probably the distal portion of the convoluted tubule or Schaltstück, which contains no blue staining granules, the argentophile bodies are in the form of rounded globules or granules, varying slightly in size and intensity of staining, and occupying a zone in the middle nuclear plane (fig. 22B). The homology of these granules to the Golgi apparatus is not clear, and it is possible that they represent excretory substances of some sort, possibly chlorides or phosphates (Leschke (109)).

Finally, in still another portion of the tubules (corresponding to the Henle loop) there is obtained an excellent impregnation of the basal filaments and mitochondria (fig. 22 C). The cytoplasm in the supranuclear zone is somewhat more intensely stained,

but no definite structures comparable to the Golgi apparatus of other cells is brought out.

This description corresponds to the appearances usually observed in the frog's kidney. Some of our preparations, however, show curious structures of very different type, the nature of which is entirely obscure. These are limited to certain portions of the secretory tubules—probably the distal convoluted portion or Schaltstück, although it is not possible to be sure of this.

They consist of sheaves of filaments, often of great length, sometimes beaded or with nodular varicosities. They run either perpendicular to the basement membrane, along the lateral aspects of the nucleus, or in some instances, lie above the nucleus and have a course more or less parallel to the basement membrane (figs. 20–21).

These bundles of fibrils do not appear to form closed skeins or to anastomose with one another, but overlies and cross. The individual fibrils are often irregularly fusiform, with tapering ends. They may be quite rigid, almost crystalline in appearance, or more wavy and filamentous. Scattered amongst them are small isolated clumps and granules of varying size.

b. In ordinary connective tissue cells. A small juxta-nuclear apparatus has been described by v. Bergen (10), Cajal, Deinecke (47) and Kolster (102); in endothelial cells by v. Bergen (10) and by Cajal (28); in smooth muscle cells of blood vessels by v. Bergen (10); and in various types of wandering cells by v. Bergen (10), Verson (179) Maccabruni (115), Barinnetti (6) (Plasma-cells—relation to centrosome, '12) and Fananas (55).

In *cartilage cells*, Pensa (136) first described a distributed apparatus which he considered to resemble the diffuse net found by Golgi in the ganglion cell. Later v. Bergen showed that the Golgi apparatus in cartilage cells, as in most other non-nervous elements, was limited to the juxta-nuclear region, and interpreted Pensa's diffuse apparatus as a chondriom. Pensa (137) has accepted this correction, and v. Smirnow (165), Barinnetti (6) and Kolster (102) are in agreement with v. Bergen on this



Fig. 21 Frog's kidney. Tubules showing argentophile filaments. Golgi.

Fig. 22 Frog's kidney. A—Proximal convoluted tubule. Golgi apparatus in the form of discrete peri-nuclear filaments, rings and loops. B—Tubule containing argentophile droplets. C—tubule showing impregnation of basal-rods.

point. Comes (40, 41) still claims that Golgi net and mitochondria in cartilage cells are identical structures.

The controversy is interesting, because it has brought out the point that the silver method is not always specific, and that under certain conditions the mitochondria may be impregnated. This occurs regularly, as I have mentioned, in the Henle tubules of the kidney.

Both Pensa (136) and Cajal (31) have described an interesting series of changes in the zone of growing cartilage adjoining the line of ossification. Following the enlargement of the cells the net loses its localized character, hypertrophies and finally, with the degeneration of the cartilage cell, undergoes granular disintegration.

Cajal (31) seems to have been the only one to study the behavior of the Golgi apparatus in *osteoblasts*. During the period of functional activity, the net is very well developed, and large, usually occupying that part of the cell directed towards the osteoid tissue. In the finished bone corpuscle, the net shrinks to a small compact mass.

Teeth. Although Massenti (123), using pig embryos, had previously described a Golgi apparatus in the form of a large skein occupying almost the entire cytoplasm of the pulp cells and odontoblasts Cajal (31) depicts structures of a more typical character and localization. As the odontoblast becomes differentiated from the connective tissue elements of the pulp, the net increases in size, and comes to form a large oval rather granular mass, occupying the supra-nuclear portion of the cytoplasm. The further fate of the structure could not be followed, since decalcification interferes with the reaction, but Cajal regards the hypertrophy during the secretory phase of the odontoblast as another example of the cyclical changes seen in goblet cells, growing cartilage cells, etc.

Muscle fibers. There are a number of papers dealing with the endo-cellular reticulum of striated muscle fibers. The earliest is that of Cajal (26), in which he found in the wing muscle of certain insects a network continuous with the ramifications of the tracheal tubes, and therefore probably a true cana-

licular system. This was confirmed by Fusari in 1894 for mammalian muscle and by Veratti (178) in 1902. Veratti, however, denied that the endocellular apparatus in insects represented a continuation of the tracheal tubes, and regarded it as composed of solid filaments. Sanchez (155), a pupil of Cajal, confirmed the tracheal origin of the reticulum in insects, looking upon it as a tubular apparatus, probably of importance for the nutrition of the fibers. The system is composed of transverse meshes on either side of the 'bandes claires,' united by longitudinal connections.

Two other recent papers, one by Martinotti (122), the other by Fananas (54) have not been available.

Quite different is the apparatus described by Luna (113) in the cardiac muscle fibers. Here he finds granules, rods, curved filaments or more complete nets lying at one or both poles of the nucleus. He believes that the granules and rods might possibly be mitochondrial.

c. Gonads. The studies upon the gonads are of much more theoretical interest, because of their bearing upon the question as to whether the Golgi apparatus is a permanent structure, and a heritable constituent of the cytoplasm, or whether it is more ephemeral in character, and related to the vegetative activities of the cell. It is also in the sperm cells that the topographical relation of the net to the centrosphere is most evident, so that the structure during spermatogenesis might be expected to show interesting modifications.

Platner (145), a number of years before the discovery of the Golgi apparatus, had described a structure surrounding the centrosomes of the spermatogonia, which he called a Nebenkern. Heidenhain (68) in 1900, in the sperm cells of *Proteus*, found with the iron hematoxylin stain, an incomplete basket-work surrounding the centriole. Heidenhain used the term *Zentralkapsel* or pseudo-chromosomes for these bodies, and believed that they were formed by special differentiation from the Benda mitochondria. This view finds support in the recent observations of Chambers (33) upon spermatogenesis in the grasshopper, in

which the mitochondria, vitally stained with Janus green, appeared to enter directly into the formation of the Nebenkern.

Sjövall (164), using a modification of the Kopsch method, demonstrated these structures in the spermatocytes, spermatogonia and spermatids of the white mouse, and concluded that Heidenhain's view of their mitochondrial origin was erroneous. Benda, and especially Weigl (180), have also taken this stand. Sjövall also found a net in the Sertoli cells.

Perroncito (140, 141), using the Golgi method, carried the observations of Sjövall a step further, by describing the alterations of the net during the maturation divisions. He finds in *Paludina*, that in the prophase, the net breaks up into granular fragments, which he calls dittosomes, and which are distributed equally to the two daughter cells. The skeins are then reformed from the granules, and in the spermatids come to occupy their usual juxta-nuclear position. This process of fragmentation and distribution during mitosis he calls dittokinesis.

The fate of the Golgi apparatus in the adult spermatozoa is unknown, nor has it been established that the substance of which it is composed enters the egg during fertilization. The phenomenon of dittokinesis, however, is established, not only for the sperm cells, but also for the somatic cells (fig. 4). Deinecke (47) ('12) has given a very clear description of this process in the flat cells of Descemet's membrane, where the net forms in the resting cell a thick skein of interwoven and anastomosing threads. During karyokinesis the skein becomes looser and gradually surrounds the nucleus, the individual threads grow thicker, loose their connection and break up into unequal bent fragments which are heaped up at the poles of the nucleus. During anaphase they change into short thick rods and granules surrounding the nuclei, and lying chiefly in the equatorial plane. The size of these dittosomes is only slightly smaller than that of the chromosomes, their number somewhat greater. With the formation of the diaster, the dittosomes surround the daughter chromosomes, being more densely aggregated at both poles. The region of the spindle remains free of them.

The new nets are formed by a fusion or sticking together of the granules or rods, but may remain discreet for a time. In this way, it is possible to recognize a recent mitosis, even after the nuclei have reformed.

During the monaster stage one sees often a pairing of the granules and double rods. Whether this indicates a splitting of the dittosomes comparable to that of the chromosomes, Deineke leaves undecided. At any rate, the above series of changes unquestionably leads to an even distribution of the mass to the two daughter cells.

Studies of the Golgi apparatus in the female gonads have been made by Sjövall (164), Weigl (180), Cattaneo (32), and Kulesch (104), and Weigl (180) and Hirschler (70) in the oocytes of invertebrates; in the primitive germ-cells of 3-4 day chick embryos by v. Behrenberg-Gossler (16). All these writers are in substantial agreement as to the main facts namely, that in the young oocytes and in the follicle cells, there is a circumscribed net at one pole of the nucleus, which in the ripe ovum breaks up into filaments and granules (or, according to Kulesch, small irregularly angular rings, bent threads and discs) which are with difficulty distinguished from the mitochondria and other cytoplasmic granulations.

V. THE GOLGI APPARATUS IN EMBRYONAL CELLS

The recognition by Golgi (64), Fananas (54) and Cajal (31) that the Golgi apparatus is present in all types of cells, even at a very early stage of development (chick embryos of 30-40 hours—Cajal) seems to establish firmly the principle that the structure is an important and constant component of the cell.

In many of these fetal cells—the mesenchyme—the endothelium of the pericardium and of the primitive blood spaces—the cells of the Wollfian ducts and the entoderm of the intestinal tract, the neuroblasts, and even the erythroblasts and wandering cells, the apparatus is highly typical and constant in its relation to the centrosphere.

The attempt has been made by Fananas to trace the development of the net from granules and batonnets in the cytoplasm.

As Cajal (31) points out, however, the not infrequent impregnation of the mitochondria and of skeletal and sustentacular structures in early embryonic cells makes such an interpretation doubtful.

One general principle can be deduced from a study of the Golgi apparatus in embryonic tissue, and that is the definite polarity of the structure in all fixed non-mobile cells. The location of the net in every case, as Cajal has pointed out, is such that it occupies the 'external' part of the cell,—that is, the portion above the nucleus directed originally towards the free surface, and opposite that pole which is towards the interstitial tissue and the nutrient supply. This polarity is preserved in adult life in the case of epithelial cells lining ducts and cavities, but may be lost in the case of solid glandular organ or tissues which undergo profound modification and derangement during development. The significance of this polarity which would seem to be bound up with the relation of the Golgi net to the centrosphere, is by no means clear, but it seems to be one of the most fundamental and most striking characteristics.

VI. THE GOLGI APPARATUS IN PROTOZOA

The literature contains but one reference to the occurrence of the Golgi apparatus in Protozoa. Hirschler (70) describes in the cytoplasm of *Monocystis ascidia*, a Gregarine parasite of the ascidian *Ciona intestinalis*, diffusely scattered ring and half ring forms, demonstrable by prolonged exposure to 2 per cent osmic acid and resistant to turpentine (Sjövall's modification of the Kopsch method). Whether these structures are the homologues of the Golgi apparatus of the metazoan cell, needs further study.

VII. THE GOLGI APPARATUS UNDER PATHOLOGICAL CONDITIONS

The modifications which the Golgi apparatus undergo under pathological conditions have been little studied, and, so far, have not added any new suggestions as to the real nature of the structure.

In the cells of malignant growths, Golgi nets, often atypical, have been found by Moriani (125) in a human breast carcinoma, by Veratti (177) in a transplantable mouse cancer; by Lucioni (112) in a naevus; by Savagnone (157) in carcinoma of the breast, in a sarcoma of the jaw and in a giant-celled sarcoma; by Tello (172) in carcinoma and adenoma, in epithelioma and in experimental granuloma caused by Kieselguhr injections. Tello studied especially the distribution of the net in foreign body giant cells, where there are multiple nets—one usually in relation to each nucleus. In tuberculous giant cells, on the other hand, as shown by Fananas (55), the net is usually centrally placed, in relation to the multiple centrosomes.

Regressive changes (fragmentation, pulverization, etc.) in the Golgi net have been described by Fananas (55) in caseating giant cells; by Marcora (117) in the ganglion cells of the hypoglossal nucleus, following avulsion or section of the nerve; by Battistessa (8) in the ganglion cells of animals poisoned by lead or strychnin; by San Giorgi (156) in toxic nephritis, and by Del Rio Hortega (97) in the ganglion cells of a case of paralytic rabies.

Very interesting are the recent experimental studies of Cajal (31) dealing with the effect of traumatic injury of the nerves upon the Golgi apparatus.

An incision of the central nervous tissue brings about complete destruction of the net only in those ganglion cells most severely injured by the trauma. The apparatus of cells near the line of incision, though perhaps slightly compressed or deformed, shows no grave disorganization. This would indicate a considerable fixity of structure, and firmness of texture, since, were the impregnated substance of fluid consistence, one would expect to find it dispersed through the cytoplasm or confluescing into larger droplets.

Cajal has further established the fact that section of a peripheral motor nerve had no effect upon the structure of the Golgi apparatus in the central ganglion cell. There does occur a degeneration of the apparatus in the cell of the sheath of Schwann, distal to the section.

There are thus very few controlled studies of the alterations of the net under experimental conditions, and it seems that something further should be added to our knowledge in that way. The great difficulty had been, and will be, the capricious behavior of impregnation methods; until simpler and more reliable methods shall have been discovered, the interpretation of slight variation in the morphology of the structures will always be open to considerable suspicion.

VIII. GENERAL CONSIDERATIONS

This completes the list of cells and tissues in which structures of this type have been demonstrated. They may be considered as universally present in every type of cell, although the variety of form which they assume at once brings up the query as to whether they are all homologous structures. That, I think, is almost impossible to answer until we know something of their function and significance. Morphologically there seems to be no single character by which we can group them altogether. The staining reactions are probably not entirely specific; we have found instances—as for example in the Henle tubules and in cartilage cells—in which mitochondrial structures are more or less regularly impregnated by the silver methods.

In many types of cells in which the location of the centrosome is known, there is, as Barinetti (6) insists, a topographical relation between Golgi net and cytocentrum. But such a relation cannot be established for the ganglion cells, in which the net completely encircles the nucleus, nor for the muscle cell, nor for the cells of the choroid plexus, still less for the cells of the convoluted tubules, or for the liver cells. So that this criterion is not universally applicable, at least to fully differentiated and highly specialized types of cells.

Much of the discussion about the nature and homologies of these things has hinged about the question as to whether they are solid, that is fibrillary; or canals filled with fluid, and made to appear as solid filaments by the metallic impregnation methods. With the canalicular theory the name of Holmgren is associated, and though there are many shades of opinion in

regard to detail the general idea that these nets and filaments represent canals with or without definite walls, has had the support of such expert histologists as Studnicka (170), Retzius (149), Cajal (31), and, in this country, of Bensley (14) and Cowdry (42). Holmgren was not the first to observe 'endocellular canals' or 'Saftkanälchen.' As far back as 1887 Nansen (129) described in the protoplasm of nerve cells of *Homarus* and in the spinal ganglion cells of *Myxina glutinosa*, primitive tubes consisting of hyalin contents enveloped in sheathes of spongioplasm. These were probably identical with the Saftkanälchen. Nelis (132) also described an 'état spirémateux' in the cytoplasm of certain mammalian nerve cells. He speaks of 'bandes incolores' of about the same diameter, sometimes straight, sometimes convoluted which did not branch, and therefore formed no reticulum. This spireme was not constantly found, and does not seem to be the same thing as the Golgi net.

Holmgren (73) made his first contribution to this subject in 1899, a year after Golgi's first paper, and without, apparently, knowing of the work of Nansen and Nelis. In the spinal ganglion cells of rabbits and of *Lophius piscatorius* he found an endocellular system of canals communicating with the pericellular lymph spaces; and in the following year he published a large monograph on the ganglion cells of *Lophius* (72). His earlier views, expressed in these papers, that the endocellular Saftkanälchen are to be regarded as lymph channels, and are thus continuous with structures of connective tissue origin, were later abandoned by him.

Morphologically Holmgren considered his canaliculi to be identical with the Golgi net, basing his opinion upon a study of his own preparations, and those of Retzius, prepared according to the earlier Golgi technique.

In 1900 (76), in a study of the ganglion cells of *Helix pomatia* Holmgren described a penetration of the nerve cells by cell-processes from surrounding cells. This view was developed in subsequent studies. The penetrating cells of neuroglial origin he called trophocytes, and to the network formed by the penetrating cell processes, he applied the term trophospongium.

These prolongations of the trophocytes, he believed, became canalized by a vacuolization of their cytoplasm, the confluence of the vacuoles giving origin to the canal. This was an irreversible process, but the cell prolongation was capable of amoeboid motion within the host cell.

Studies on various tissue cells, which I shall not review in detail, confirmed him in his view, and lead him to the following generalization. The cells of the body are of two orders of physiological dignity—high and low. Those of exalted function are the nerve cells, muscle cells, sex cells, certain glandular cells. The lower order of cells, which are the trophocytes, function as servitors, looking after the wants of their more specialized neighbours by means of their trophospongia.

Although this hypothesis is vaguely expressed, and open to obvious criticism, Holmgren has maintained it in a long series of papers (77-96), many of them controversial, and adding no new evidence. The arguments against any such generalized conception are apparent enough. To what order shall we assign the leucocytes and other wandering cells? Where are the trophocytes of cartilage cells? Why have the trophocytes about the ganglion cells, as well as all the other types of cells classed with the lower order, endocellular nets, and what cells look after their lowly wants?

Holmgren has always insisted vigorously upon the identity of his trophospongium with the Golgi apparatus; on the other hand, all who have worked with the Golgi or Cajal methods deny that the structures which they bring out reach the surface of the cells or communicate with other cells. Even Cajal, who regards the Golgi structures as canaliculi, believes them to be wholly endocellular, except perhaps in the special case of the insect muscles, in which the homology with the Golgi net is not very clear at best.

Ross (153) in a recent paper on the trophospongium of the ganglion cells of the crayfish, describes the penetration of the cytoplasm by partitions and fibrils from the surrounding neuroglia, but rejects the idea that these have any relation to the internal reticular apparatus.

This view, I think, may be accepted without reserve; nor does it seem that Holmgren's generalizations are based on sound evidence, nor that they have added much of value to the subject.

Leaving aside, then, this controversial phase of the subject, one may ask what can be said as to the more intimate physical structure of the Golgi apparatus. It seems to the writer, that the conceptions of Cajal (31) best meet the observed and established facts. Cajal's view is that the apparatus represents a canalicular system, filled with a lipoid-containing substance which reacts to the specific impregnation methods employed. The walls of this system are presumably fairly fixed and rigid in cells of a sedentary habitus, and permanent form, but more plastic in secretory cells and in young cells frequently undergoing mitosis.

It seems probable that the quantitative changes observed during activity indicate a using up of a store of stainable material within these canaliculi, and that the re-appearance of the net during the quiescent stage is due to the re-accumulation of the substance within more or less preformed and permanent channels. What purpose this material serves in the cell metabolism, and what is its more intimate chemical structure, are questions unanswerable with the data at hand.

IX. SUMMARY AND CONCLUSIONS

There is present in the somatic and sex cells of all metazoa, and possibly also, of protozoa, a cytoplasmic structure of considerable complexity and size, demonstrable by prolonged fixation in osmic acid, or by silver impregnation and reduction. The reaction of this structure to osmic acid indicates, of course, a lipoid component, but there are no other data bearing upon its chemical composition. Nor is anything certain known of its physical characters. Its invisibility in the living cell would indicate a low refractive index. The fundamental question as to whether the impregnated structures are canalicular or filamentous remains unsolved. The constant topography in many types of cells, particularly the definite relation to the cytocentrum would favor the idea that the structures are at least in part

solid, rather than casual rifts or fluid-filled canals in the cytoplasm. The fragmentation or dispersion of the net which occurs during certain secretory phases, or accompanying pathological changes in the cell, and particularly during cell division, would also suggest a solid or semi-solid consistence.

The Golgi apparatus in the secretory portion of the renal tubules does not conform to the usual closed skein found in many types of glandular epithelial cells, but is dispersed and assumes complex and varying forms. In the glomerular cells, on the other hand, and in the epithelium of the collecting tubule and of the pelvis the structure is more typical both in form and location.

Injury to the epithelial cells of the convoluted tubules (uranium nitrate poisoning) is followed by complete disintegration and disappearance of the Golgi apparatus. This appears to precede the complete necrosis of the cell. The large hyalin droplets found during the degeneration of the cells contain an argentophile component possibly derived from the remains of the Golgi apparatus.

The structures brought out by the Golgi or Cajal technique are more resistant to autolysis than are the mitochondria.

There occurs regularly in the rat's kidney an elective impregnation of the mitochondrial filaments of certain portions of the Henle loops. This illustrates the fact that the method is not absolutely specific.

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ON THE FORM AND ARRANGEMENT IN FASCICULI OF STRIATED VOLUNTARY MUSCLE FIBERS

A PRELIMINARY REPORT

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FOUR FIGURES¹

In the last few decades, relatively little special attention has been given to the form of striated voluntary muscle fibers and to their arrangement in the fasciculi. In the anatomic literature of this period, consideration is given mainly to the structure of the myofibrils, to the relation of the connective tissue of the muscle to the fibers, to the development of muscle fibers and the development of the musculature as a whole. One studying current texts is impressed with the unanimity of expressed views concerning the form of muscle fibers and the question is treated as having received satisfactory solution.

Heidanhain¹ in 'Plasma und Zelle' treats of the form and length of voluntary muscle fibers as follows:

Da der Gegenstand allgemein bekannt ist, können wir uns kurz fassen. Es handelt sich um lange faserförmige Gebilde, welche in kleinen Muskeln von einem Sehnenende bis zum anderen hindurchlaufen und an diesem immer abgestumpft enden, im Inneren sehr grosser Muskeln hingegen auch frei und zwar unter allmählicher Verschmälung mit spitzen Enden auslaufen. Sie werden bei geringer Breite (9–60 μ) bis zu 12 cm. lang und daher findet man nur in Muskeln, welche, parallel der Faserung gemessen, die Ausdehnung von 12 cm. überschreiten, die erwähnten freien Endigungen.

This quotation expresses fairly well, I believe, the current views of the form and mode of ending of striated voluntary muscle fibers.

¹ Heidanhain, M., *Plasma und Zelle*. Fischer, Jean, 1911, p. 529.

Bardeen,² as a result of a study of teased preparations made from the external oblique of certain mammals, deviates from the current views and gives a much more correct statement as concerns the form and relations of striated voluntary muscle fibers; indeed his brief statement is one of the most accurate I have found and is here given in full. His words read as follows:

The individual muscle-fibres either run from one tendon to another or they may end at one extremity or at both within the muscle fasciculi which extend from tendon to tendon. We may therefore distinguish two modes of ending of individual muscle-fibres: the '*intratendinous*', where the tip of the fibre terminates within a definite extension of a well marked tendon; and the '*intrafascicular*,' where the muscle-fibre terminates in the midst of a bundle of other muscle fibres which have a different region of termination. In the former case the muscle-fibre has a rounded or cone-shaped termination, often swollen in isolated specimens. In the intrafascicular mode of ending the muscle-fibre gradually becomes more and more narrow until it terminates in a thread-like extremity.

In Bardeen's figures 2 and 4, types of these modes of ending and of the form of muscle-fibers are given, figure 4 including spindle-shaped fibers. Bardeen's statement of the form and mode of ending of muscle fibers, however, is antedated by the account given by v. Kölliker in his *Handbuch der Gewebelehre des Menschen*, which may be added to the references here given. Kölliker's³ account reads as follows:

Ueber die Gestalt der Muskelfasern haben besonders die Untersuchungen von Herzig und Biesiadecki, dann von mir, W. Krause, Weismann, Aeby und Kühne Aufschluss gegeben. Nach diesen Erfahrungen kann es wohl als Regel bezeichnet werden, dass die Muskelfasern im Innern grösserer Muskeln spindelförmig sind, die an den Enden dagegen ein inneres spitzes und ein in die Sehne übergehendes breites Ende besitzen, welches entweder abgerundet ist oder in einige stumpfe Spitzen ausläuft oder auch wie treppenförmige Absätze darbietet. Ausser spindelförmigen Fasern kommen ein Innern der Muskel noch manche andere Formen vor, am gewöhnlichsten an dem einen oder an beiden Enden stumpfe Fasern.

² Bardeen, C. R., Variations in the internal architecture of the m. Obliquus Abdominis Externus in certain mammals. *Anat. Anz.*, vol. 23, 1903.

³ v. Kölliker, A., *Handbuch der Gewebelehre des Menschen*. Erster Band. Engelmann, Leipzig, 1889, p. 371.

Kölliker quotes E. H. Weber (the original I have been unable to find) as regarding spindle-shaped fibers as the prevalent form of striated voluntary muscle fibers.

It is not my purpose at the present time to enter upon a more extended discussion of the literature dealing with the form and arrangement in fasciculi of striated voluntary muscle fibers. It is hoped that the quotations given may suffice to orient the results here to be presented. In the course of this brief report other pertinent literature will be considered as occasion demands.

My own studies on the form of striated muscle fibers have been made largely on teased preparations. The muscular tissue was obtained largely from adult rabbits. The maceration preparatory to teasing was by the hydrochloric acid method developed in this laboratory.⁴ The method as used for the maceration of muscular tissue may be given here in detail in the hope that other workers may feel tempted to make use of it in a further analysis of this tissue, since a correct understanding of the form and arrangement of muscle fibers in fasciculi, as also their length, is of importance in valuating certain fundamental conceptions concerning the functions of muscles. For instance, according to E. Weber's law of the working capacity of a muscle we are taught that the lifting power of a muscle is proportionate to the cross section of its fibers or fasciculi when arranged parallel, while the extent of elevation is proportionate to the length of its fibers.

The method as used is as follows:

After freely bleeding an adult rabbit a cannula was inserted into one of the iliacs central to the inguinal ligament or into the subclavian before it passes under the clavicle and firmly secured by ligature. A 75 per cent solution of hydrochloric acid was then quickly injected at a pressure of 25 to 30 pounds. The apparatus used in obtaining and maintaining pressure was described by the author in the *Am. Jour. Anat.*, vol. 6.⁵ It is desirable to have the acid injected enter the tissues as quickly and as freely as possible. The pressure is maintained for several minutes. Some 15 to 20 minutes after the injection is com-

⁴ Huber, G. Carl, A method for isolating the renal tubules of mammals. *Anat. Rec.*, vol. 5, 1911.

⁵ Huber, G. Carl, The arteriolae rectae of the mammalian kidney. *Am. Jour. Anat.*, vol. 6, 1907.

pleted, the muscles are exposed and separated and removed and placed in a 75 per cent solution of hydrochloric acid. In removing a muscle care should be taken to remove the entire muscle, at least portions extending from tendon to tendon and great care should be taken not to crush the muscle during removal. The muscle pieces or entire muscles remain in the hydrochloric acid for about 3 hours, the period varying a little, depending on the thoroughness of the preliminary injection. After thorough maceration is obtained, the acid is carefully poured off and distilled water slowly added. The water is renewed at frequent intervals until it is practically free from acid. In the distilled water the muscle pieces remain about 24 hours, though a stay of 4 to 6 days is not harmful. After thorough washing in distilled water the larger pieces are usually readily broken up into smaller bundles of fasciculi. Small bundles of fasciculi are now transferred to a hemalum solution diluted to one half with distilled water. The transfer from the distilled water to the hemalum solution should be executed with care if one wishes to obtain fasciculi through their entire length. The transfer is best made with a glass rod, lifting the small bundle carefully as it leaves the water. In the hematoxylin solution the small bundles remain about 24 hours. This interrupts the maceration and stains the fibers. They may be kept indefinitely in the hematoxylin solution and are best stored in this solution for future use. In this solution the muscle bundles become quite hard and brittle and contract to about two-thirds or even to one-half of their former length. The preliminary teasing I have carried on in Esmarch dishes under the stereoscopic binocular and in a 0.5 per cent solution of ammonia water. In the ammonia water the stain attains a purple-blue color and the hard and brittle bundles become soft and pliable. A stay of one half hour to one hour in the ammonia water prepares the bundles for preliminary teasing. In the Esmarch dishes the bundles of fasciculi are with care separated into separate fasciculi. It should be stated, however, that a fasciculus is not a unit of muscle structure. For a distance all of the fasciculi of a bundle are readily separated. However, at one or several points small bundles of fibers or single fibers pass from one fasciculus to contiguous fasciculi. Great care is thus necessary and very careful teasing to completely separate what is known as a fasciculus. Bardeen² has noted the fact that muscle fasciculi are joined by fibers. After the preliminary teasing resulting in the separation of a single fasciculus, the final teasing is undertaken on a large slide or lantern slide cover prepared as follows. The slide is thoroughly cleaned in acids and alcohol and wiped dry. Narrow strips of wax plates (the plates used in wax reconstructions) 2 mm. thick are cut and placed near the borders of the slide in the form of an oblong and pressed to the slide. The slide is then gently heated until the wax strips adhere. The slide on cooling is ready for use. The shallow well thus formed is filled with ammonia water and an isolated muscle fasciculus transferred to it. The final teasing may then be undertaken. It has repeatedly been possible to separate completely all or nearly all of the muscle fibers of a given fasciculus, even with fasciculi having a length of about 6 cm. The teased fibers may then be arranged in their approximate positions. Only when this has been accomplished can a muscle fasciculus be considered as having been teased. A worker should not attempt this unless he has at his disposal some 3 to 4 uninterrupted hours, and ought to bear in mind that the best results are obtained by 'making haste slowly.' The mounting of such preparations presents many difficulties and discouragements. My procedure

is as follows: A fasciculus is teased until nearly all its fibers have been separated. The ammonia water is then very slowly and carefully withdrawn by means of a small dropper with point drawn to a capillary tube. This is undertaken under the binocular, observing the effects of currents. The water is withdrawn until only a thin layer remains, only sufficient to enable moving the fibers on the slide. The final teasing and arranging of fibers may now be undertaken. As the ammonia water evaporates, the muscle fibers begin to adhere to the slide. The wax wall may now be removed and a large cover glass, on the under side of which a thin layer of glycerin has been spread, is gently lowered over the preparation from one edge. It is necessary to obtain the right degree of drying in order to gain successfully mounted preparations. If not sufficiently adherent to the slide the muscle fibers will move, float and break. If allowed to dry too much the muscle fibers, although fixed in place, will appear fragmented. Such preparations are not valueless since the fragments of the fibers are not displaced laterally. Thus a single fiber may readily be traced throughout its whole length.

By this method of preparation the muscle fibers show only faint cross striations, though they present a blue color. The nuclei are not evident. Neither has it been possible to locate the place of entrance of the nerve fibers. The sarcolemma seems very resistant to the acid, the neurolemma less so. In the ammonia water the muscle fasciculi appear to regain the length they had prior to staining in the hematoxylin solution. The exact relation existing between the lengths as presented by teased fibers and living muscle fibers I am unable to determine definitely.

The drawings here presented were made from preparations of muscle fasciculi teased completely, and arranged on the slide in their approximate positions, approximate with reference to the ends of the fasciculus teased. The drawings were made with the aid of the camera lucida at a magnification of 50 diameters, and are reduced 10 times in reproduction. The length of the respective fibers is accurately given. The thickness of the fibers is correctly given as pertains to the thicker portions of the fibers. At the attenuated ends the ink lines follow the outer border of the pencil outlines.

The results of these observations may be briefly recorded as follows:

It is usually stated that in muscles having relatively short fasciculi the muscle fibers extend from tendon to tendon. This is of course not determined by the size and length of the muscle as a whole since in semipinnate, pinnate and compound pinnate muscles and in muscles where distal and proximal tendons overlap the lengths of the respective fasciculi of a given muscle are much shorter than the length of the muscle as a whole.

In figure 1 are presented some muscle fibers teased from fasciculi taken from the gastrocnemius of an adult rabbit. The fibers in group A, drawn from a completely teased fasciculus taken from the proximal portion of this muscle have an actual length of 1 cm. (these and all measurements given are obtained by dividing the length of the respective fiber as measured in



Fig. 1 Muscle fibers from the gastrocnemius of an adult rabbit. Group A, actual length 1 cm., from fasciculus taken from the more proximal portion of the muscle. Group B, actual length 1.5 cm., from fasciculus taken from the more distal portion of the muscle. $\times 5$.

the drawing by 50, the drawing having been made at a magnification of 50 diameters). The fibers in group B have an actual length of 1.5 cm. and are from a completely teased fasciculus taken from the more distal part of the same muscle. In all of the fasciculi from this muscle completely teased, the great majority of the muscle fibers extend from end to end or from tendinous insertion to tendinous insertion. In material prepared as above described, at the place of termination of a muscle fiber

in tendon, the end of the muscle fiber stains more deeply in hematoxylin than does the same fiber in close proximity. The true end of the fiber differs in appearance from the end of a broken fiber. The tendon ends of various fibers vary slightly in shape. They may appear as if cut at right angle to the fiber, as slightly beveled, as slightly rounded, tapering a little or having the form of a blunt cone and now and then as slightly expanded, though this may be due to a slight flattening of the end of the fiber. Now and then tendon ends of muscle fibers are met with that give the impression as though the sarcolemma did not enclose the end but terminated ring-shaped at the extreme tendon end of the respective fiber, but the limitations of the method used are such that this question could not be conclusively decided. In this connection it is of interest to note the observations of O. Schultze,⁶ who believes that muscle fibrils and tendon fibrils are parts of a single structure but this observer adds that the behavior of the sarcolemma at the ends of the fibers deserves further study. Also the studies of Baldwin,⁷ who regards the sarcolemma as covering the tendon ends of muscle fibers and denies the continuity of muscle fibrillae and tendon fibrillae, and discusses two types of terminations of muscle fibers in tendon; one type in which the long axis of muscle and tendon fibers coincide, the other type in which they meet at an angle. In the former the tendon fibrils are attached to cone shaped processes of the sarcolemma dovetailed into the tendon ends; in the latter type the sarcolemma end is considerably thickened and presents a number of projections into the muscle substance. Digitations or branchings of muscle ends or step formations have not been observed by me in my teased preparations. It should be understood, however, that in successfully macerated preparations the collagenous connective tissue is so completely removed that it is not evident on teasing. Out of quite a number of fasciculi with fibers of type B, of figure 1,

⁶ Schultze, O., Über den direkten Zusammenhang von Muskelfibrillen und Sehnenfibrillen. Arch. f. Mik. Anat., vol. 79, 1912.

⁷ Baldwin, W. M., The relation of muscle fibrillae to tendon fibrillae in voluntary striped muscle of vertebrates. Morph. Jahrb., vol. 45, 1913.

successfully and completely teased, in only two and in each only one fiber was found which did not extend from tendon end to tendon end. In both of these fibers one end reached the tendon, terminating as adjacent fibers, while the other end reached to about the middle of the fasciculus ending in a fine tapering filament. The fibers in a number of fasciculi having muscle fibers of the length of type B were counted and averaged about 20 fibers to a fasciculus.

What length a muscle fasciculus of an adult rabbit may attain and still have the great majority of its fibers reach from end to end is a question I am at present unable to answer definitely. Of the muscles teased, none in which the contained fasciculi reached a length of a little over 2.5 cm. did I find such in which the majority of the muscle fibers reached from end to end. However, samples have not been taken from nearly all of the muscles and it may be that in certain of them fasciculi having a length of over 2.5 cm. in which the majority of the fibers extend from end to end, may be found.

In figure 2 are presented type fibers obtained from a completely teased and successfully mounted fasciculus, taken from one of the adductor muscles of the thigh of an adult rabbit. In this fasciculus a single muscle fiber (A) extends from end to end or from tendon insertion to tendon insertion; both extremities showing the characteristic staining and appearance of the tendinous end of a muscle fiber. This fiber has an actual length of 3.64 cm., a length which is regarded as the length of the fasciculus. After final teasing and after withdrawing the ammonia water

Fig. 2 Muscle fibers from the thigh adductor of an adult rabbit. Teased fasciculus had a length of about 3.5 cm. The completely teased fibers are in the drawing placed with reference to the ends of the fasciculus. Fiber A, has actual length of 3.64 cm.; a, 2.1 cm.; b, 1.8 cm.; c, 1.5 cm.; d, 1.3 cm.; e, 2.1 cm.; f, 1.9 cm.; g, 1.7 cm. $\times 5$.

Fig. 3 Types of muscle fibers teased from a single muscle fasciculus, having a length of a little over 4 cm., taken from one of the larger thigh muscles of a rabbit. This fasciculus contained 37 fibers. The fibers are arranged with reference to an imaginary line, bottom of figure. The tendon ends of fibers ending intrafascicular are brought to this line. Certain of the fibers sketched have an actual length as follows: a, 2.9 cm.; b, 2.4 cm.; c, 1.92 cm.; d, 0.9 cm.; e, 1.4 cm.; f, 0.14 cm.; g, 2.9 cm.; h, 3.04 cm.; i and j, 2.9 cm. $\times 5$.



from the well on the slide, as explained in the detailing of the method used, I was able to arrange the teased fibers so as to have the tendon ends of the teased fibers reach imaginary lines drawn at right angles to the ends of the single muscle fiber which extends from end to end in this fasciculus. The spindle-shaped fibers hold approximately the same relative position with reference to the ends of the fasciculus as before teasing as was determined at the time of teasing. This fasciculus, completely teased, contains 26 muscle fibers, of which as stated one passes from end to end, 10 others reach one tendon end, 12 the other tendon end and 3 are spindle shaped fibers reaching neither tendon end. Of the 26 fibers, 15 type fibers are given in figure 2. This bundle of fibers completely teased is here spoken of as a fasciculus. I have above stated that fasciculi are not units of structure, but that from each small bundles of fibers or single fibers pass from one fasciculus to contiguous fasciculi. A single 'fasciculus' completely separated constitutes thus an artificially separated bundle of muscle fibers. Thoma⁸ has also appreciated the fact that a muscle fasciculus is not a unit of structure. In serial cross sections of the gastrocnemius of the frog, in which, with the aid of the camera lucida, the outlines of the muscle fibers were sketched serially he noted single muscle fibers passing from one muscle fasciculus to another, concluding as follows: "Die einzelnen Muskelfaserbündel hängen somit vielfach durch Muskelfasern zusammen, welche bald mit dem einen, bald mit dem anderen Bündel sehr innig verbunden sind, und die ganze Muskelmasse bildet ein stark in die Länge gezogenes Netzwerk von Muskelfasern." The fasciculus above referred to as containing 26 fibers is to be considered in this light. In the figure as drawn, at each end 5 fibers begin with blunt ends showing by form, structure and staining that they are muscle fibers ending in tendon. Each of the fibers extends into the fasciculus for a distance which varies for the several fibers, becoming attenuated and finally terminates in a thread like filament having a thickness of $3\ \mu$ to $4\ \mu$. It requires very

⁸ Thoma, R., Über die netzförmige Anordnung der quergestreiften Muskelfasern. Virchow's Archiv, vol. 191, 1908.

thorough maceration to enable one to separate completely these fine, intrafascicular terminations of the muscle fibers. The length of the muscle fibers having one tendon end at the end of the fasciculus, the other ending in an intrafascicular filamentous termination varies as follows; fiber a, 2.1 cm.; fiber b, 1.8 cm.; fiber c, 1.5 cm.; fiber d, 1.3 cm. The other fibers of this type sketched are intermediate in length between fibers a and d. The three spindle shaped intrafascicular fibers with both extremities attenuated and neither end reaching the tendon ends of the fasciculus measure as follows, fiber e, 2.1 cm.; fiber f, 1.9 cm.; and fiber g, 1.7 cm. The extent of overlapping of fibers beginning at the tendon end of the fasciculus and ending intrafascicular in fine, attenuated ends may be noted in this figure (2). Their exact relation cannot be readily seen in a completely teased preparation, with fibers separated and arranged on the slide. To gain their relationship actual teasing is necessary. While teasing the details of the arrangement of the muscle fibers becomes evident and it is observed that the fine filamentous intrafascicular ends are applied usually to the thicker portions of other fibers, usually not near a filamentous end of another fiber. The same is true of the ends of the spindle-shaped fibers reaching neither fascicular end. This figure (2) I regard as representative of the form and arrangement of the striated voluntary muscle fibers in the fasciculi of rabbit muscles having a fascicular length of from about 3 cm. to about 5 cm. Probably the same is true of voluntary muscle of other vertebrates, though my observations have not been extensive outside of rabbits and birds (rooster).

In muscle with longer fasciculi the length of the muscle fibers having blunt tendon ends and filamentous intrafascicular terminations varies more than indicated by the measurements above given, and the spindle shaped fibers with intrafascicular position may lie nearer one end or the other of the respective fasciculus or occupy a more middle position. This variation in the length of the muscle fibers I have indicated in figure 3, giving type fibers from a fasciculus having a length of somewhat over 4 cm., and taken from one of the thigh muscles. Un-

fortunately the specific muscle could not be determined after the maceration. This fasciculus was also completely teased and successfully mounted. It contains 37 fibers of which 8 are spindle shaped and have an intrafascicular position. In it one fiber extends from end to end, through the length of the fasciculus. The fibers could readily have been sketched in approximate relative position with reference to the ends of the fasciculus, but the resulting figure, at the magnification used, would have been too long to admit of publication in the pages of this Journal. The arrangement of the fibers, however, is not unlike that presented in figure 2. For figure 3, type fibers were selected. The single fiber extending from end to end could not be included by reason of its length. The fibers having a tendon end are arranged with reference to an imaginary line, at the bottom of the figure; the tendon end being brought to this line. Of certain of the fibers with tendon ends and intrafascicular filamentous terminations the actual lengths are as follows, fiber a, 2.9 cm.; b, 2.4 cm.; c, 1.92 cm.; d, 0.9 cm.; e, 1.4 cm.; f, 0.14 cm. The spindle shaped fibers sketched with both ends terminating intrafascicular with filamentous endings present the following measurements, fiber g. 2.9 cm.; h, 3.04 cm.; i and j, 2.9 cm. The single fiber extending through the entire fasciculus presents a length of almost 4.5 cm.

For rabbit muscle fasciculi having a length of more than 4.5 cm. to about 5 cm., so far as my observations go, there are no muscle fibers that extend the whole length of a respective fasciculus. In some of the longer fasciculi taken from the latissimus dorsi, the pectoralis major and the extensor cruris almost complete teasing was obtained. Many muscle fibers were completely isolated, though never all of the fibers of a given fasciculus. In some of the most successfully macerated fasciculi, their distal ends were slightly crushed during removal, so that not all of the fibers could be traced to their tendinous ends. For final teasing of these longer fasciculi, lantern slide covers answer the purpose of slides very well. In the longer fasciculi, having a length of 6 cm. to about 6.5 cm., in which many fibers were completely isolated, no fibers were found

reaching from end to end. Fibers with blunt tendon ends and filamentous intrafascicular terminations, these, severally of varying lengths, and spindle shaped fibers with intrafascicular position, with ends terminating in hair like processes, constituted the types of fibers isolated. In these longer fasciculi one end of certain of the spindle shaped fibers reaches nearly to one or the other tendinous end of the respective fasciculus while others of the spindle shaped fibers have a more nearly central position, with reference to the length of the fasciculus. In the longer and longest fasciculi teased, no muscle fibers having a length of more than about 3.5 cm. were observed.

Felix⁹ is quoted as having isolated striated muscle fibers approaching a length of about 12 cm. In his account stress is laid on the fact that in the macerating fluids used, acids mainly, the muscular tissue contracts by one-third to two-thirds of the original length. In his own material he sought to obviate this contraction by maintaining the original length through tension. I have noted the fact that in the method used, hydrochloric acid is injected into the living muscle while under extension, that during immersion in the hydrochloric acid and in the hematoxylin stain, a contraction of the muscle fasciculi to about two-thirds to one-half of their original length is obtained, but also that in the ammonia water fasciculi of muscle taken from the hematoxylin solution extend in length so as to approach very nearly their length in fresh muscle. Exact measurements I am unable to give since, obviously, it would be necessary to isolate at least small bundles of fasciculi from fresh muscle, and trace them through the various steps, making measurements at various stages. Of the longest fibers isolated by Felix, one from the gracilis of man measured 11.5 cm. and one from the sartorius of man 12.3 cm.; the latter fiber having a broken end. Division of fibers was not seldom found. A figure of a single fiber with branchings is reproduced natural size. This fiber in the figure measures approximately 12 cm. Concerning this fiber the text speaks as follows: "Die Faser theilt sich, lässt

⁹ Felix, W., Die Länge der Muskelfaser bei dem Menschen und einigen Säugethieren. Festschrift, Albert von Kölliker, Englemann, Leipzig, 1887.

Spalträume erkennen, steht mit anderen Fasern in Verbindung, kurz um, das Bild wird durch vielfach abgehende Fasern ein so complicirtes, dass man ein Gewirr von mehreren Fasern vor sich zu haben glaubt, bis eine genaue mikroskopische Untersuchung ihre Zusammengehörigkeit nachweist." Felix teased unstained tissue. I have not teased human muscle. However, the figure presented by Felix is not unfamiliar to me. In incompletely macerated tissue such 'fibers' are now and then obtained. However, they are interpreted by me as representing an incompletely teased fiber complex. The fine hair like intrafascicular ends of muscle fibers are so closely applied to the sides of other fibers that the cross diameter of the thicker fiber is scarcely increased. Such a misinterpretation, I can conceive, may readily be made in incompletely macerated and teased muscle tissue. Felix gives data concerning the length of muscle fibers in the rabbit, a tissue with which I am familiar. This observer isolated fibers from the pectoralis, sartorius, latissimus dorsi and extensor cruris of the rabbit. His own words concerning them read as follows: "Hier waren fast sämtliche Fasern mindestens 5 cm. lang, doch waren unter 6 cm. nur wenige zu erzielen. Die meisten Fasern schwankten zwischen 6.0 und 7.5 cm. Die Fasern zeichneten sich sämtlich durch ihre Stärke aus. Die längste Faser isolierte ich aus dem extensor cruris, der am Thiere selbst nur 8 cm. mass, von 8 cm. Länge. Die Dicke war ungemein schwankend, dickere und dünnere Stellen wechselten ab, die dünnste Stelle mass nur 0.0109 mm., während dickere Stellen 0.111 mm. gemessen wurden. Offenbar sind hier verschiedene Wirkungen der Salpetersäure zur Geltung gekommen. Theilung konnte ich häufig beobachten."

An analysis of this statement from Felix in the light of my own investigations leads me to conclude that this observer did not obtain completely teased muscle fibers. Many hundreds of muscle fibers of the rabbit have been completely isolated and in no instance have I observed branching of fibers. Often have I seen apparent branching, but on careful teasing such structures have been separated into several fibers. The variation in thickness of the long fibers referred to in the above quotation, I be-

lieve, is explained by a linking in chain of several fibers. Even granting that the fasciculi teased by me after a stay in ammonia water, some attaining a length of about 6.5 cm., had not attained their full, original length, the difference in the length of muscle fibers of the rabbit teased by Felix and by myself is not accounted for. Felix found few attenuated ends of fibers with intrafascicular terminations, while, as my own figures show, these are numerous. In the light of these studies I am inclined to regard the measurements of the length of striated voluntary muscle fibers as given by Felix as inaccurate and as made on incompletely teased muscle tissue, and to regard the figures given by earlier observers as more accurate. These, to quote freely from Felix, are for the medium length of muscle fibers of man 2 cm. to 3.5 cm., Krause giving as the longest of the fibers of the sartorius 4 cm.

Striated voluntary muscle fibers of other mammals and other vertebrates have thus far been only incidentally teased by me. Bardeen's² figure 4, b, gives a flat band of fibers dissected from the external oblique of a dog, having a length of approximately 15 cm. (figure one-half natural size) with figures of completely isolated fibers; spindle shaped fibers having a length of approximately 8 cm. and fibers with blunt tendon ends and attenuated intrafascicular terminations, varying in length from approximately 4 cm. to 6 cm. The general shape of these fibers appears to me as correctly drawn. Since I have not teased muscle fasciculi of the dog I am unable to verify the accuracy of the measurements given. For the dogs muscle fibers Felix gives 3 cm. to 4.5 cm. as common measurements and 5.5 cm. to 6.5 cm. as long fibers.

Opportunity presented itself to tease muscle fibers of an adult rooster (*Gallus domestica*), injected with hydrochloric acid for other purposes. In one specimen, the thigh muscles were well macerated. In figure 4 are shown four completely teased spindle shaped fibers taken from these muscles. These fibers, some of which are among the longest completely teased, present the following measurements: fiber a, 3.2 cm.; b, 3 cm.; c, 3.2 cm. and d, 2 cm. Several spindle shaped fibers with intra-



Fig. 4 Spindle shaped muscle fibers teased from the thigh muscles of an adult rooster (*Gallus domestica*). Actual length of fibers, a, 3.2 cm.; b, 3 cm.; c, 3.2 cm.; d, 2 cm. $\times 5$.

fascicular position, with undoubted branching were observed. The division extended to about the middle of the respective fibers, the two parts terminated in attenuated, hair like fibers. Muscle fibers with blunt tendon ends and filamentous intrafascicular terminations were also observed.

It is the purpose, as opportunity presents, to include in this study fibers from different types of muscles from the different classes of vertebrates and to extend the investigation so as to include several different mammals with types of muscle from each.

Schiefferdecker¹⁰ and certain of his pupils have spent infinite pains in determining, among other things, the relative thickness of muscle fibers. The thickness and form of muscle fibers these workers have determined largely in cross sections of various muscles. Each muscle is said to be composed of muscle fibers having specific size and form (cross section) with specific arrangement of connective tissue and elastic fibers. It is recognized that in each muscle, muscle fibers of varying sizes are found. In many muscles this difference in size of fibers is said to be considerable, in others less so. This difference in size of fibers may be ascribed, according to Schiefferdecker, to two possibilities: 1, the muscle may be composed of fibers which in reality differ in size; 2, the smaller and smallest cross cut fibers of a given cross section may represent cross sections of the ends of fibers terminating in the muscle. In considering the structure of muscle, he adds, the second possibility plays only an unimportant rôle, and only as concerns the smallest fibers. The fibers sketched in figures 2 and 3 may serve to show that such contention is difficult to support in the light of this work. Except for muscles in which the fibers of the respective fasciculi extend from end to end, or in which the majority of the fibers do this, the variation in the size of the fibers in a given cross section is largely dependent on the fact that many of the fibers of a given fasciculus do terminate intrafascicularly. In order to make the numerous measurements of Schiefferdecker and his pupils of real value, or of similar investigations, it would be necessary to ascertain by means of teasing and complete isola-

¹⁰ Schiefferdecker, P., *Muskeln und Muskelkerne*. Barth, Leipzig, 1909.

tion of fibers, the arrangement of the fibers in the fasciculi of muscles, the fibers of which are measured in cross sections.

MacCallum's¹¹ investigations led him to conclude, as a result of counting the fibers of the sartorius muscle in man at various ages that the muscle fibers cease to multiply in the fetuses from 13 cm. to 17 cm. in length, and that after that period muscles increase in size by increase in size of individual fibers. This statement, it would seem to me, needs verification and could only be verified by study of muscles in which all of the fibers of the fasciculi extended from end to end or by very careful and painstaking teasing, of fasciculi, covering the several periods in which the muscle fibers are counted.

Myofibrils are usually regarded as extending from end to end in a given muscle fiber. In muscle fibers having filamentous intrafascicular terminations, and this includes the majority in the longer fasciculi, this is obviously not the case. Concerning the relations of the ends of myofibrils not reaching the ends of the respective muscle fibers, my teased preparations give no evidence. The festooning of the sarcolemma, described by certain authors, may perhaps be brought in relation with the ends of myofibrils which do not extend the entire length of the muscle fiber.

In this communication the expression "completely teased and isolated muscle fibers" has been repeatedly used. Therefore it will no doubt seem paradoxical, for me to express in this concluding paragraph, even tentatively, the view that striated voluntary muscle is syncytial in character.

From the arrangement of muscle fibers in the fasciculi of striated voluntary muscle; from the fact that muscle fasciculi are not units of structure; from the further fact that in teasing muscle fibers there are always found points of contact where the fibers are ultimately separated with great difficulty, I am led to tentatively express the view that striated voluntary muscle tissue presents syncytial character even in its fully developed state, as does involuntary muscle and cardiac muscle, though

¹¹ MacCallum, J. B., On the histogenesis of striated muscle fibers and the growth of the human sartorius muscle. Johns Hopkins Bull., 1898.

not to the same degree as the last named. This question cannot be finally decided by teasing. It is not my purpose at the present time to enter upon the mooted question of the histogenesis of voluntary muscle tissue, nor to consider the extensive literature involved. The problem of the syncytial character of voluntary muscle is one of histogenesis. Embryological evidence at hand indicates that the histogenesis of voluntary muscle lends support to the view that striated voluntary muscle is syncytial in origin. Material is being collected to determine this question if possible. One of Schiefferdecker's¹⁰ general conclusions reads as follows: "Muskelnetze fanden sich in den untersuchten Muskeln so vielfach, dass man sie wohl als eine allgemein verbreitete Erscheinung ansehen kann." Thoma⁸ finds frequent anastomoses between fibers. Reference, however, is not had to anastomoses between fibers such as described by Thoma. This observer finds intimate contact between adjacent fibers, so that for a distance only a single layer of sarcolemma appears to separate them. Myofibrils are not thought to pass from one fiber to another. It has seemed to me that this may be verified in teased preparations. Now and then two fibers adhere together, for a short distance, so closely, that separation, even in well macerated tissue, is impossible; this very generally in thicker portions of fibers. Involuntary muscle, if successfully macerated in potassium hydrate or by the hydrochloric acid method here detailed is readily teased so as to present spindle shaped cells, although as shown by McGill¹² this muscle develops from mesenchyme, retaining its syncytial character. The mere arrangement of striated, voluntary muscle fibers in a fasciculus possessing fibers with attenuated intrafascicular terminations, is such as to suggest the syncytial character of this tissue. In partially teased, though well macerated tissue, a mesh work of fibers, with long meshes is now and then evident. It is usually possible to tease the fibers having intrafascicular termination, quite readily, so far as concerns the thicker portions of these fibers and to isolate them to near their thread like terminations

¹² McGill, Caroline, The histogenesis of smooth muscle in the alimentary and respiratory tract of the pig. *Monatschrift Anat. u. Phys.*, vol. 24, 1907.

on other fibers. Near their intrafascicular ends they adhere very tenaciously to adjacent fibers. In ammonia water the macerated and stained fibers become quite pliable and present an elasticity and a tensile strength which is often surprising. Yet, often the finer ends are broken before they can be detached from adjacent fibers. It is evident that the relations of the intrafascicular ends of muscle fibers to adjacent fibers is different at their attenuated terminations than in course. Their exact relation I am unable to determine in teased preparations, though even the finest ends often present the appearance of a torn sarcolemma which does not extend to the extreme tip. I am unable to state whether the myofibrils extend from the attenuated ends to fibers on which they appear to end. In a number of preparations of rabbit embryos of the tenth day, cut serially in the sagittal plane, sections having a thickness of $2\ \mu$ and $3\ \mu$, stained in iron-lac-hematoxylin, the syncytial character of the cells from which the voluntary muscle tissue is developing is evident. Conclusive preparations, from embryos varying in ages, have thus far not been obtained. This question shall form the subject of a further study now under way. It may be recalled here that Godlewski¹³ considers striated muscle as presenting a syncytial character, basing his deductions on a study of the histogenesis of skeletal and heart muscle.

It is impossible at the present time to do more than suggest that striated voluntary muscle, like involuntary and cardiac muscle, presents a syncytial character, evidence of which is seen in its full development.

¹³ Godlewski, E., Die Entwicklung des Skelet- und Herzmuskelgewebes der Säugethiere. Arch. f. Mik. Anat., vol. 60, 1902.

A NOTE ON THE STRUCTURE OF THE ELASTICA INTERNA OF ARTERIES

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ONE FIGURE

A comparison of a number of texts, descriptive of the structure of the elastic intima—the fenestrated membrane of Henle—of arteries, reveals the fact that the views concerning the structure of this layer are by no means unanimous. Schäfer¹ speaks of the elastic intima as follows: “The elastic tissue is represented by one distinct lamina, which is separated from the endothelium by the subendothelial layer. It is, on its outside, in direct contact with the non-striped muscle of the middle layer.” The ‘internal elastic lamina’ is spoken of as membranous in character, the membrane is not, however, a continuous one, but is perforated by apertures. In figure 517, of Schäfer’s text, is shown a portion of the fenestrated membrane from the femoral artery as figured by Henle. Mall² in his study of connective tissue fibrils states that “elastic fibers are composed of two distinct substances—the interior, which stains intensely with magenta, and the membrane, which does not.” A study of the membrane of Henle, isolated by boiling in acetic acid or KOH and then stained with magenta or picrocarmine leads him to conclude that “The Henle’s fenestrated membrane is therefore composed of three layers—an upper and a lower transparent membrane in which there are no openings, and which is identical with the membrane of elastic fibers; and a middle layer which stains with magenta, and is identical with the interior of elastic

¹ Schäfer, E. A. Text book of microscopic anatomy. Longmans, Green, and Co., 1912, p. 332.

² Mall, F. P. Reticulated tissue, and its relation to connective fibrils. Johns Hopkins Hospital Reports, vol. 1.

fibers. This central portion contains the openings." V. Ebner's³ description of the elastic intima concludes as follows, "Uebrigens erscheint dieselbe fast immer als eine sogenannte gefensterte Haut mit verschiedenen deutlich ausgeprägten, netzförmigen Fasern und meist kleinen länglichen Oeffnungen, seltener als ein wirkliches, aber sehr dichtes Netz vorzüglich langsverlaufender elastischer Fasern mit engen, länglichen Spalten, und stimmt in ihrem chemisches Verhalten vollkommen mit den elastischen Häuten der Media grosser Arterien überein." Triepel⁴ in his account of the elastic tissue in the walls of intracranial arteries considers the elastic intima as a fenestrated membrane, stating that in the smaller arteries the fenestra are so near together that only a felt-work of elastic fibers remains, so that in cross sections a row of adjacent points is observed instead of a membrane. His figure 4 shows this clearly. Schöppler,⁵ who studied the finer structure of the brain arteries of several mammals, gives especial consideration to the internal elastic membrane, and gives emphasis to closely arranged longitudinal ledges, which have a course parallel to the long axis of the vessels. He recognizes a fibrillar structure in the elastic intima as expressed in these words, "Vielfach zeigt sich auch, dass die Membrana flava interna keine homogene Platte ist, sondern wie die Betrachtung von Schrägschnitten bei 1000facher Vergrösserung lehrt, aus, sehr feinen elastischen Fäserchen besteht. Die erwähnten Leistchen werden durch Ausbildung stärkerer nach dem Lumen vorspringender Fasern bedingt." His figure 6, which shows an oblique longitudinal section of a basilar artery presents the fibrillar character of the elastic intima clearly. Dürck⁶ records observations made on connective tissues studied by means of Weigert's iron-hematoxylin myelin sheath staining method. In tissues fixed in formalin and Müller's fluid or in formalin,

³ v. Ebner, Victor, Kölliker's Handbuch der Gewebelehre des Menschen, Dritter Band, Zweite Hälfte. Engelmann, Leipzig, 1902, p. 643.

⁴ Triepel, H. Das elastische Gewebe in der Wand der Arterien der Schädelhöhle. Anat. Hefte, vol. 7, 1897.

⁵ Schöppler, H. Ueber die feinere Struktüre der Hirnarterien einiger Säugtiere. Anat. Hefte, vol. 15, 1900.

⁶ Dürck, H. Ueber eine neue Art von Fasern im Bindegewebe und in der Blutgefässwand. Virchow's Archiv, vol. 189, 1907.

mordanted in a copper salt and stained in iron-hematoxylin, following the Weigert method for staining myelin sheaths, certain connective tissue fibrils were stained blue-black. Certain of these differentially stained fibrils were regarded by Dürck as a special type of connective tissue fibrils, others, as yellow elastic fibers. This method as used by this observer, gave, in successful preparations, unusually distinct staining of the elastic intima of vessels. His words read as follows, "Untersucht man zunächst kleine Arterien auf dem Längsschnitt oder auf Schrägschnitten, welche das Rohr in langer Ausdehnung treffen, so erkennt man an den durch die Intima fallenden Schnitten, dass die Elastica interna hier nicht durch zirkuläre Fasern, Faserbündel oder Lamellen dargestellt wird, wie man dies gewöhnlich abgebildet und beschrieben findet, sondern unmittelbar über dem Endothelrohr liegt wie eine Basthülle unter einer Baumrinde eine einfache Schicht von straffen Längsfasern, welche unter sich allerdings durch kurze quere Zwiechenstücke verbunden sind und so ein Netz mit sehr langgestreckten und längs verlaufenden Maschen darstellen." In cross-sections such fibers appear as points.

The method used in staining the sections on which this study was based and from one of which the figure accompanying this note was drawn, was presented by Dr. De Witt⁷ at the Wisconsin meeting of the American Association of Anatomists in 1907. This differential elastic tissue staining method consists of a modification of Weigert's⁸ iron-hematoxylin van Gieson method. According to Weigert's method two stock solutions are prepared.

Solution I

Hematoxylin crystals.....	1 gram
Alcohol, 96 per cent.....	100 cc.

Solution II

Liquor ferri sesquichlorati (U. S. P.).....	cc.
Hydrochloric acid (sp. gr. 1.20).....	40
Aqua dist.....	7
	950

⁷ DeWitt, Lydia M. Abstracts of papers presented at the 22nd Session Amer. Ass. Anat. Anat. Record, vol. 1, p. 74.

⁸ Weigert, K. Eine kleine Verbesserung der Hämatoxylin-van Gieson-Methode. Zeitsch. f. wissensch. Mik., vol. 21, 1904.

Solutions I and II are mixed in equal proportions just before using. Differentiation is obtained by means of van Gieson's picric acid fuchsin solution, prepared after Weigert as follows:

	cc.
Picric acid, saturated aqueous solution.....	100
Acid Fuchsin (Weigert), 1 per cent aq. sol.....	10

In the method as used for elastic tissue staining, stock solutions I and II are mixed in proportion of 3 to 4 parts of solution I to one part of solution II. The sections are stained several hours and after rinsing in distilled water differentiated in van Gieson's picric acid, acid fuchsin solution, prepared as above indicated. The differentiation is controlled from time to time, under the microscope. The method is simple and can be used on celloidin sections or paraffin sections fixed to the slide. The yellow elastic fibers are stained blue-black, the collagenous tissue is stained brick-red to pink, depending on the degree of differentiation and the thickness of the sections. The method is not unlike that used by Dürck, although the differentiation by means of the van Gieson picric acid, acid fuchsin solution has the advantage of counterstaining the collagenous tissue. This differential elastic tissue staining method has been extensively used in the preparation of sections for classes and is recommended as simpler than other differential elastic tissue stains.

Numerous sections of arteries varying in size from arterioles with two or three layers of muscle cells to arteries of about 2.5 mm. in diameter, cut in pieces of tissue fixed in formalin, formalin and Müller's fluid, and picro-nitric solution, embedded in paraffin, and sections fixed to slides, were stained after the above mentioned iron-hematoxylin and picric acid, acid fuchsin method. The differentiation in picric acid, acid fuchsin was carried in most sections to an extreme degree, so that only the yellow elastic tissue retained any of the blue-black coloring. Usually four to six sections were fixed to one slide, the sections approximating 5μ in thickness. They were cut on the sliding microtome, thus varied a little in thickness and gave slightly varying degrees of differentiation. The larger and largest arteries were, owing to want of suitable material, not included in this special

study, though previous incidental study of such vessels leads me to believe that the elastic intima, where present as such, is in general character like that of the smaller vessels. In no instance were the arteries especially studied removed from the surrounding connective tissue, so that the staining of the elastic tissue in the perivascular areolar tissue served as a control for the staining of the elastic tissue in the arterial walls.

In all of the successfully stained preparations and in arteries varying in size from the smaller to the larger ones studied, the elastic intima appears, when successfully differentiated, as a



Fig. 1 Elastic intima of deep plantar artery, human. Stained in iron-hematoxylin and van Gieson's acid fuchsin, picric acid solution. $\times 600$.

network of elastic fibers, the larger fibers of the network having in the main a direction which is parallel to the long axis of the respective vessel. A well stained and well differentiated longitudinal or longitudinal oblique section of an artery including the elastic intima, appears not unlike a successfully teased preparation of yellow elastic tissue from the ligamentum nuchae.

In figure 1 is presented a drawing of a portion of the elastic intima of one of the larger deep plantar arteries of a human foot. During fixation the artery had collapsed in such a way that on one side, for a distance, its wall was nearly in a plane. Several sections of a series thus included long stretches of the elastic intima. In this figure only the elastic tissue, which is

stained deeply blue-black, is reproduced as drawn with the aid of the camera lucida, using a $\frac{1}{12}$ inch oil immersion objective and a No. 4 Zeiss compensation ocular with paper at table level. The network character of the coarser elastic fibers with frequent anastomoses and numerous cross-bridges is faithfully reproduced. It was not possible to draw accurately all of the finest fibrils throughout their entire extent. However, the figure as a whole gives a correct impression of the appearance presented by the section. At both ends (above and below the figure), the intima leaves the plane of section and the elastic fibers, shown as a network in the figure, appear as cross cut or obliquely cut fibers. In numerous other sections of vessels of varying sizes, longitudinally or obliquely cut, including the elastic intima, similar appearances are found. The character of the network varies but slightly, dependent on the degree of extension or distension of the respective vessel. Oblique sections approaching cross sections of vessels are especially instructive. In such sections a side view of the elastic network of the elastic intima with end view of the fibers as seen in cross-cut, is obtained by moving the micrometer screw of the microscope. In cross sections of vessels, in place of the usual line representing the elastic intima as seen after the usual staining, there is observed a row of deeply stained blue-black dots, varying in size with the size of the vessel, with here and there a longer or shorter blue-black dash where a cross anastomosis between fibers is included in the section.

Sections of areolar connective tissue, differentially stained for elastic tissue by means of the iron hematoxylin picric acid, acid fuchsin method, present no evidence of an 'outer membrane' for elastic fibers as described by Mall. However, the existence of such a membrane is in no sense denied, since a slight tinging with picric acid would not be evident against the deep blue-black stain of what is probably the 'inner substance,' stained readily in magenta. In certain of the longitudinal sections of vessels including the elastic intima, as for instance in the section from which the figure here presented was drawn, a delicate grey-blue color overlies the elastic network. This is represented in the figure by a light wash of neutral tint. If

this be expressive of structure it reveals a homogeneous structure and may possibly indicate the presence of a homogeneous membrane. Such a membrane, however, I have not detected in cross sections of vessels.

From this study of the elastic intima of arteries the conclusion seems warranted that the stainable substance of this layer consists of a network of yellow elastic fibers, with coarser fibers having in the main a course which is parallel to the long axis of the respective vessel, these fibers presenting frequent anastomoses and cross bridges, and with numerous finer fibrils which pervade the network. Here and there certain of the fibers of the elastic intima may in cross or oblique sections be traced in anastomosis with elastic fibers of the media. It would thus appear desirable to discard the term 'fenestrated membrane,' since this term does not express the structure of this layer. Of previous descriptions, that given by Dürck appears to me the most nearly conforming with observed facts.

A NOTE ON THE MORPHOLOGY OF THE SEMINIFEROUS TUBULES OF BIRDS

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ONE FIGURE

In the course of a study of the renal tubules of birds, by means of the maceration method devised by Huber,¹ in which full grown roosters (*Gallus domestica*) were used as material, the injection of the 75 per cent solution of hydrochloric acid was through the aorta central to the branches supplying the kidneys and sex glands. In a number of the cases the testes were found to be well injected and were removed and placed in 75 per cent hydrochloric acid with a view of obtaining thorough maceration preparatory to teasing. Following the method as described, the macerated pieces were washed thoroughly in distilled water, stained in hemalum, softened and cleared in 0.25 per cent to 0.5 per cent ammonia water, in which they were teased.

Even during the preliminary teasing of the larger pieces it was noted that the testis of the rooster was not separable into lobular masses, as is the case in mammalian testes, so that it was found impossible to isolate structural units with which the final teasing could be carried out.

Huber and Curtis² found that in the mammalian testis the seminiferous tubules presented no blind ends, diverticuli or nodular enlargements but were arranged in the form of an arch or a variable number of linked arches, all of the ends terminating

¹ Huber, G. Carl. A method for isolating the renal tubules of mammalia. *Anat. Rec.*, vol. 5, 1911.

² Huber, G. Carl, and Curtis, George Morris. The morphology of the seminiferous tubules of mammalia. *Anat. Rec.*, vol. 7, 1913.

in tubuli recti attached to the rete testis. Wax reconstructions made by Curtis confirm the observations made on teased preparations. Repeated teasings have convinced me that in the adult bird no such arrangement of tubules pertains, but that the seminiferous tubules of the bird are arranged in the form of a network, presenting a varying number of anastomoses found at different levels in the gland substance. For this reason the teasing of these tubules is exceedingly difficult in that it is impossible without breaking or tearing tubules to separate favorable pieces preparatory to final teasing. All of my teased preparations present an endless net, with broken tubular ends as a boundary.

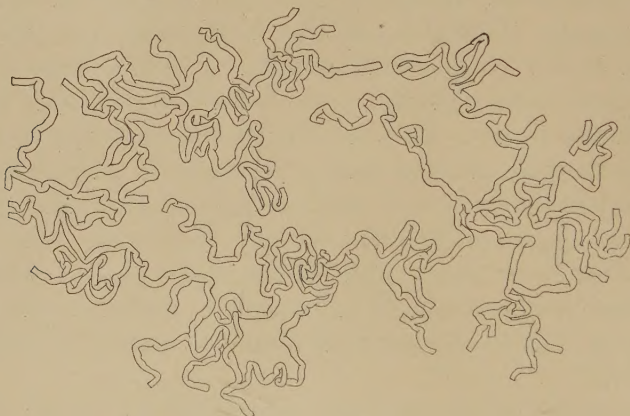


Fig. 1 Teased preparation showing a portion of the tubular system of the testis of the bird (*Gallus domestica*). $\times 5$.

In the accompanying figure is presented one of the most successful preparations obtained. The figure was traced and sketched with the aid of the camera lucida at a magnification of 50 diameters, reduced to a magnification of 5 diameters in the reproduction. The portion of the tubular net reproduced presents in all some forty broken ends, and at least three closed rings. Such closed rings I have found in nearly all of my preparations. Their complete separation requires great care and patience since uniting tubular portions are very easily broken. The clearness of the figure, it is thought, obviates the necessity

of extended description of the character of the network formed by the seminiferous tubules of birds. The figure, however, should be studied with the understanding that in the mount from which the figure was drawn the teased tubules were spread out as much as possible. The figure, therefore, does not give spatial relations of the tubules. In the gland, as is well known, these tubules form compact coils, evident somewhat from the extended kinks and bends seen in the figure.

In a rather careful search of the literature I have been unable to find any description of the form of the seminiferous tubule of birds. This note would thus seem justified. However, the observations here recorded would seem to me to have a bearing on previous work emanating from this and other laboratories, relating to the form of the seminiferous tubules of mammalia. The results here recorded seem to me to confirm the observations made on teased preparations of the seminiferous tubules of mammals. The fact that complex anastomoses resulting in closed ring structures have been teased in the bird's testis argues for the possibility of teasing such structures in the adult mammalian testis, did they exist. Bremer,³ as a result of careful wax reconstructions of the tubular system of the human testis, working on embryonic and fetal tissue, the oldest stage studied being that of a human fetus of seven months, reached the conclusion that

The testis cords, growing from the germinal epithelium of the genital ridge, form a network with three sets of anastomosing branches. After completion, this network breaks down partially, leaving certain cords as persistent stems. The tubules of the adult show, in their course, connection, and position in the testis, traces of this network. Testis tubules may be single, ending blindly, may branch, or may anastomose.

In the adult mammalian testis tubules, completely teased, no blind endings, buds, nor ring formations were observed, while in the teased preparations of the seminiferous tubules of birds, the remains of the network of tubules as observed in the embryonic and fetal stages and well figured by Bremer, may be

³ Bremer, John Lewis. The morphology of the tubules of the human testis and epididymis. *Amer. Jour. Anat.*, vol. 11, 1911.

noted. In a cryptorchid of the rabbit, as described by Huber and Curtis, extended anastomoses of testis tubules were observed in two regions of the tubule complex, and in two regions, near the periphery of the gland, tubules were joined so as to form two folded rings. The preparations from the cryptorchid of the rabbit present appearances not unlike those shown in teased preparations of the seminiferous tubules of the bird. The presence of the remains of the embryonic network of the seminiferous tubules in the cryptorchid of the rabbit and in the bird's testis, postulates a relatively late, complete morphogenesis of the seminiferous tubules of the mammal. Phylogeny and ontogeny indicate this. In the light of these observations I am of the opinion that Bremer's careful study of the morphology of the seminiferous tubules of the human testis are of value as concerns embryonic and late fetal stages, but may not be transmitted to the adult gland in that in the oldest stage studied, a human fetus of the seventh month, the seminiferous tubules, in all probability, had not completed their morphogenesis. The question is one deserving further study and will form the subject of a future, more comprehensive communication, based on especially prepared and 'timed' material from the rabbit. This form is chosen since the morphology of the seminiferous tubule of the adult rabbit has received special consideration in this laboratory, both by means of teased preparations and reconstructions.